PCT / IB 99 / 00522
11. 06. 99
09/647004

REC'D 14 JUN 1999
WIPO PCT

Kongeriget Danmark

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

Patent application No.:	0424/98
Date of filing:	25 Mar 1998
Applicant:	Steen Østergaard Skovlyporten 10,st.4 DK-2840 Holte

The attached photocopy is a true copy of the following document:

- The specification, claims and and drawings as filed with the application on the filing date indicated above.

Patentdirektoratet · Kongeriget Danmark ·

Erhvervsministeriet
Patentdirektoratet

TAASTRUP 03 Jun 1999

Lizzi Vester

Lizzi Vester
Head of Section

MANIPULATION OF PARTICLES

The present invention concerns manipulation of particles by using fields to which the particles are susceptible, primarily for analytical purposes or for performing synthesis. In particular, particles that are susceptible to magnetic fields are considered.

Background

The automation of reagent handling is a central issue in the development of systems for chemical analysis and synthesis. Higher degree of automation expands the possibility of chemical analysis and increases the availability of chemical information. The benefit of this comes to medical diagnostics, environmental monitoring, process control and other disciplines, that require detailed chemical information. Typically, reagent handling is automated either by robotics, by pressure driven flow systems or by systems based on electrophoretic forces.

Miniaturisation added to the automation gives advantages as better flow control (pressure driven systems work exclusively in the laminar flow regime), increased efficiency of chromatographic processes (e.g. capillary electrophoresis) due to increased surface-to-volume ratio, reduced reagent costs, and the possibility to make portable, sensor-like systems. The acronym " μ -TAS" (micro total analysis system) is used for such systems and covers micro systems with automation of every operational step in a chemical assay. A flow injection analysis (FIA) system is another approach to miniaturised reagent handling and eventually analysis.

Controlled manipulation and measurement of magnetic particles

The key concept of the present invention is to do reagent handling by immobilising said reagent on particles and move

the particles by fields to which the particles are susceptible, such as e.g. magnetic fields, gravitational fields, an apparent field applied by centrifugation or electric fields by which the particles are moved by electrophoresis or dielectrophoresis. To control the particles, the fields has to be created with high repeatability and with a complexity that allows for particle movement within and between the compartments as desired.

By moving the reagent coated particle between and within compartments of different chemical environments in a controlled and well-timed manner, a multitude of analytical chemical operations can be performed. Said compartment may have different functions, such as sample interaction, complexing measurable probes to the bound analyte (on a particle), detection of specific properties of the particles, making derivatives of the coated chemicals (synthesis) or washing the particles.

The particles have a diameter typically in the range from 0,5 micro meter to 1 mm, depending on the process. The particles may preferably be made of a magnetically responsive material (MRM) and optionally one or several coating materials, to make the particles both magnetically responsive and suitable for reagent coating. Many different kinds of magnetic particles are commercially available from companies such as Dynal, Bangs Laboratories and Miltenyi.

Non exclusive examples of the MRM's in the particles could be alloys, oxides, sulfides and borides of iron, nickel cobalt and some rare earth elements. The MRM can either be ferromagnetic, paramagnetic or superparamagnetic. For some embodiments paramagnetic and even more superparamagnetic particles are preferably used since they loose their own magnetism as soon as the external magnetic field is removed.

Many different bulk materials for surrounding the MRM have been reported. A few examples are polystyrene, starch,

dextran and poly-methyl-methacrylate. The bulk material itself, additives or surface modifications can be used for coating the particles with reagents.

The magnetic particles can be coated with many types of reagent, for example antibodies, that will bind a certain antigen (molecular or particular); double or single stranded DNA or RNA for PCR (Polymerase Chain Reaction) or hybridisation assays; drug receptors; biotin or streptavidin; chelates; pH or red-ox sensitive indicators; precursors of a desired product for synthesis. The reagent can be bound to the particle in every way known in the art of immobilisation. For example covalent bonds, ionic interactions, and non-polar interactions or combinations thereof have been employed.

Other embodiments of the invention uses chemically sensitive, magnetically responsive eucaryote or procaryte organisms such as magneto-bacteria for magnetic particles. The microorganisms can either have a native magnetic responsiveness (magneto-bacteria or red blood cells with deoxy-haemoglobin) or have acquired the feature artificially.

When the invention is used for analysis, the analytes are strongly related to the reagent coating of the magnetic particles. Some analytes that relates to reagent mentioned before are be enzymes, other proteins, hormones, vitamins, cells, vira, drugs (are bound by antibodies or drug receptors); DNA and RNA (are bound by DNA- and RNA-probes); metal ions (are bound by chelating agents); pH and red-ox potential (from pH or red-ox indicators).

Compartments

A fluid comprised within a compartment is substantially physically unaffected by the contents and action in the adjacent compartment within a time scale relevant for the processes.

A compartment is confined from the surroundings by compartment walls except for an optional number of inlets or outlets and an optional number of interfaces that interconnect the compartment to other compartments. The compartment walls may be liquid, solid or gas phase. The inlets/outlets are meant to replenish the compartment with whatever chemical solution it needs to perform its function in the analysis/synthesis systems.

According to the invention, a typical analysis or synthesis system consists of many different compartments. Some are mentioned here as examples.

Particle storage/supply compartments

Before performing the assay or synthesis the particles are situated in compartment for particles storage. Besides the particles, the compartment may contain a pH-buffer and optional additives to assure long time stability of the reagent and the immobilisation. The pH is typically in the range 2-13, depending in the reagent, and in most cases preferably in the range 5-9.

Sample interaction compartments

During analysis the particles have to be allowed to interact with the sample and this interaction is accomplished in the 'sample interaction compartment'. The compartment typically has one inlet and one outlet for introducing the sample and two interfaces: one for the particle supply and one for a compartment of further analysis. During the sample interaction the reagents on the magnetic particles will either be chanced due to the chemical environment of the sample or bind certain analytes of the sample.

The volume of the compartment for sample interaction should have size that allows for detection of the analyte. The volume range should be within 1 pL to 10 mL.

In the case of analysis the term 'sample' is often used for an amount of liquid (or solid or gas) that is representative of the unknown liquid (or solid or gas) in the sense that the analytes from the unknown liquid should be present in sample in reproducible amounts.

One of the following reactions may happen in the compartment for sample interaction: the magnetic particles may bind analyte from the sample, the analyte may chance the surface reagents of the magnetic particles (without separating the analyte from the sample), a reaction between the immobilised reagents and the analytes takes place via an intermediate species.

Secondary interaction compartments

In the case of analysis the purpose of the secondary interaction compartment is to perform a reaction that makes the changes of the particles during sample interaction detectable. This reaction could as examples and not limitations be binding detectable probes (fluorescent antibody, fluorescent DNA, fluorescent chelates, etc.) to either unaltered particle bound reagent, analyte that is bound to particle bound reagent or particle bound reagent that has been changed due to the sample interaction.

In the case of synthesis the secondary interaction is to alter the reagents on the surface of the magnetic particle with the purpose of building up new molecules. These alterations could for example be the controlled build-up of polymers like proteins or DNA/RNA or 'point of care' synthesis of drugs or reagents.

Compartment interfaces

The interface can be one of the following: a mixing zone of the aqueous liquids of two or more compartments; a gas plug

between two or more compartments; a plug of an insoluble, organic liquid between two or more compartments; a plug of solid matter that may be melted/turned into liquid at will; an actuatable or passive membrane between two or more compartments; a breakable seal between two or more compartments.

The compartments could also be assembled or aligned just before the analysis has to be performed. One interesting embodiment of this would have a least one position for filling the compartments, one position for storing the system and one position for performing the analysis. Optionally a position for filling the sample in the system could be included.

Detailed description of the invention

One aspects of the invention concerns a method of moving a particle with at least one reagent immobilised thereon into a liquid sample that is contained in a compartment system comprising a first compartment that is interconnected with a second compartment, each of which having an opening defined therein, the method comprising the steps of

entering at least one particle with at least one reagent immobilised thereon into the first compartment,

entering the liquid sample into the second compartment,

subjecting the compartment system to a field to which the at least one particle is susceptible whereby the at least one particle is moved into the second compartment by a force exerted on the at least one particle by the field so that the reagent on the at least one particle may interact with the content of the liquid sample contained in the second compartment in such a way that substantially no convection of the liquids in the compartment system takes place during said movement and said interaction.

The invention also concerns a device comprising a compartment system in which the above described method may be carried out.

A device according to the invention comprises a system of compartments in which the content of a liquid sample may interact with at least one reagent immobilised on at least one particle, the device comprises

at least one particle with surface properties suitable for immobilising at least one reagent thereon,

at least one reagent suitable for being immobilised on the surface of the at least one particle,

a first compartment for storage of the at least one particle,

a second compartment in which the liquid sample may interact with the reagent immobilised on the at least one particle, each of said first and second compartment having at least one opening for passing liquids between the compartment and the exterior,

means for subjecting at least a part of the system to a field so as to move the at least one particle between the first and the second compartment, and

a passage defined between the first compartment and the second compartment so as to allow the at least one particle to be moved from one compartment into the other compartment through the passage.

The second compartment of the compartment system may further comprise a second opening for passing liquids between the compartment and the exterior so that the liquid contained in the second compartment may be replaced with another liquid without affecting the contents of the other compartments of the compartment system.

In a preferred embodiment, the step of subjecting the compartment system to a field comprises the step of positioning field generating means for generation of a field that is subjected to at least a part of the compartment system at the compartment system.

The step of subjecting the compartment system to a field may comprise the step of generating a magnetic field, e.g by positioning an electro magnet at the compartment system and subjecting the compartment system to a field by activating the electro magnet with an electric current.

The magnetic system may be created with one or more permanent magnets or electromagnets. The field generating means may both be integrated within the compartment system or applied externally. The magnetic field may be constant in time (e.g. stationary permanent magnet or DC electromagnet) or may be changing in time (could be due electromagnets with changing currents, moving permanent magnets or moving DC electromagnets). Zones of material with ferro-, dia-, para- or superparamagnetic can be deposited within or outside the compartment complex to focus or distort the magnetic field.

Bigger compartment dimensions, smaller particle sizes and lower content of magnetic material in the particles reduce the particle velocity. Therefore the relatively small compartments and relatively large particles will allow for a simpler and less power consuming magnetic system.

The speed of a magnetic particle in a time constant magnetic field from a permanent or electromagnet will accelerate as it approached the magnet. A substantially constant particle speed can be achieved by moving the permanent magnet or decreasing the current through the electromagnet as the particles comes closer.

In an alternative embodiment, the step of subjecting the compartment system to a field comprises the step of

positioning two electrodes in electrical contact with the liquid in the compartment system and subjecting the compartment system to a field by supplying an electric potential between the two electrodes so that the at least one particle is moved by electrophoresis.

In an further alternative embodiment, the step of subjecting the compartment system to a field comprises the step of positioning field generating means at the compartment system comprises positioning two electrodes at the compartment system in such a way that they are not in electrical contact with the liquid in the compartment system and subjecting the compartment system to a field by supplying an electric potential between the two electrodes so that the at least one particle is moved by dielectrophoresis.

The compartment system may also be subjected to an apparent field by performing centrifugation of the compartment system so as to move the at least one particle, or the compartment system may be subjected to the field of gravitation, e.g. by changing the vertical orientation of the compartment system when a movement of the at least one particle is demanded. The at least one particle may in the case of a gravitational field be moved either towards or against the gravitational field, depending on the ration between buoyancy forces and gravitational forces on the at least one particle.

Any one, alone or in combination with one or more of the other of the above-mentioned fields may also be applied to the device comprising the compartment system for enabling the steps of the method involving movement of the at least one particle. Accordingly, the at least one particle may be moved by a single or a combination of the above-mentioned fields.

The method of moving the particle into a liquid sample preferably further comprises the step of monitoring properties of the at least one particle during the interaction and/or monitoring properties of the at least one

particle after the interaction. The device according to the invention may comprise detection means for detecting properties of the at least one reagent immobilised on the surface of the at least one particle.

These properties could e.g. be fluorescent properties that may be detected with a photomultiplier or a CCD-array combined with a suitable objective or could be magnetic properties that may be detected with a Hall-sensor.

The detection of chemical properties in a compartment may employ all the methods known in the art of chemical detection. According to the invention the luminescence methods (fluorescence, phosphorescence, chemi- or bioluminescence) are specially preferred. Regarding fluorescence and phosphorescence, the compartment needs to be transparent to allow for electromagnetic excitation of the relevant molecules and getting the emitted light to the detector (assuming external detection and excitation source - these might also be integrated). Furthermore, materials of the compartment that are not autofluorescent within the wavelengths of the measured molecules are preferred when fluorescence (molecules with short-lived excited states) is detected.

The detection system may both measure the particles as they move through the detection volume or make measurements on an area where the particles are made to settle. The latter approach allows for detection with confocal fluorescence microscopy giving a high signal/noise ratio.

An alternative to the optical methods is electrochemical detection principles as conductivity, amperometry and potentiometry. External or integrated electrodes for these measurements may be used in for the detection function in a compartment. Other types of detectors such as surface acoustic wave devices may also be used.

The device with the compartment system may, according to the invention, comprise at least one field generating means adapted to apply a field to at least a part of the compartment system, and the at least one particle being at least partly made from a material susceptible to the generated field. The generated field may be a magnetic field and the field generating means may comprise at least one electro magnet and/or at least one permanent magnet.

Alternatively, the device may comprise field generating means comprising two electrodes in electrical contact with the liquid in the compartment system so that the field generating means are activated by applying an electrical potential difference between the two electrodes and the at least one particle is moved by electrophoresis.

In a further alternative embodiment of the invention, the device has field generating means that comprises two electrodes which are not in electrical contact with the liquid in the compartment system so that the field generating means are activated by applying an electrical potential difference between the two electrodes and the at least one particle is moved by dielectrophoresis.

In a still further embodiment of the invention, the device comprises field generating means that are adapted for moving the at least one particle both back and forth between compartments between which a passage is defined.

Optionally, the compartment system further comprises a third compartment for detection of properties of the at least one particle, that is interconnected with the second compartment, and the method further comprises the step of moving the at least one particle by means of the field into the third compartment so as to perform the monitoring of the properties of the at least one particle when the at least one particle is situated in the third compartment.

The optional third compartment for performing the detection of the properties of the at least one reagent immobilised on the surface of the at least one particle with the detection means may comprise

an opening for passing liquids between the compartment and the exterior,

an area that is transparent to allow of optical access from the exterior to the interior of the compartment, and

a passage defined between the second compartment and the third compartment so as to allow particles to be moved between the second compartment and the third compartment, the means for subjecting at least a part of the system to a field being adapted for moving the at least one particle between the second compartment and the third compartment by said field.

The compartment system may further comprise a secondary interaction-compartment that is interconnected with the second compartment, and the method may in this case prior to the step of monitoring further comprise the steps of moving the at least one particle by means of the field into the secondary interaction-compartment of the system, and allowing the at least one particle to interact with a liquid contained in the secondary interaction-compartment so as to make the result of the interaction between the reagents and the content of the liquid sample detectable by the detection means.

Likewise, and also according to the invention, the compartment system may further comprise a washing-compartment that is interconnected with any of the other compartments, and the method may in this case further comprise the steps of moving the at least one particle into the washing-compartment of the compartment system by means of the field, and allowing the at least one particle to interact with a liquid contained

in the washing-compartment so as to remove unwanted material from the at least one particle.

The device according to the invention may comprise any combination of one or more of the above-mentioned types of compartments.

One of the compartments of the compartment system according to the invention may optionally be adapted for letting electromagnetic radiation of certain wavelengths reach the liquid contained in said compartment for enabling a process of photoactivation to take place. The method may in this case further comprise the step of subjecting the at least one particle to electromagnetic radiation of a wavelength suitable for causing a process of photoactivation.

The at least one particle that is used in the above described devices and methods may in a preferred embodiment of the invention be of a mean diameter of 1-200 micro meter, more preferred of 1-50 micro meter and most preferred of 2-20 micro meter.

The cross-sectional dimensions of said compartments may in a preferred embodiment of the invention of the above described devices and methods be within 100-1000 micro meter, more preferred within 100-600 micro meter and most preferred within 200-500 micro meter, but at least one of the cross-sectional dimensions may also be in a smaller range, within 35-100 micro meter.

The device according to the invention is preferably manufactured from materials that are non-magnetic and/or from materials that are non-autofluorescent, such as Topas.

The compartments are preferably made from materials that do not interfere significantly with the function of the compartment. The compartment materials should not alter the contents of the compartment and not be altered itself by the

compartment contents. Typical compartment material could be polymers (milled, injection moulded etc.), glass, ceramics.

The interconnections between compartments in the device comprising the compartment system are in some embodiments of the invention closed until they by activation are opened prior to the performance of a test so as to prevent the contents of the compartments to mix before a test.

The interconnections may be closed with a material that is solid before activation, and the activation is performed by heating at least a part of the system of compartments until the closing material becomes liquid.

Alternatively, the activation of the interconnections may be performed by physically aligning the compartments.

Additional features such as electrodes for heating and temperature measurements and light guides for entering light into a compartment and getting light out from the compartment may be included.

Besides reagents the compartments may contain a wide range of additive. As an example and not limitation of the function of these additives one could mention pH-stabilisation, adjustment of viscosity, preservation (e.g. against microorganisms), adjustment of surface tension.

The invention further concerns a method of analysing the content of a liquid contained in a container, the method comprising the steps of

(a) mixing particles with the liquid so as to obtain a substantially even distribution of the particles in at least a part of the liquid, the particles being at least partly made from a material susceptible to a field, such as a magnetic field or an electric field,

and having at least one reagent immobilised on a plurality of the particles,

(b) allowing the reagent immobilised on the particles to interact with the content of the liquid,

(c) applying a field to which the particles are susceptible to at least a part of the container so as to move at least one of the particles through an opening of the container to extract the at least one particle from the container,

(d) moving the at least one particle through a liquid-filled passage to detection means for detecting properties of the reagents on said at least one particle, and

(e) detecting properties of the reagent on said at least one extracted particle in order to determine whether these properties have changed due to the interaction, so as to perform an analysis of the liquid.

The steps (c) to (e) may be repeated at least once after elapse of a predetermined time period so as to provide a monitoring of a possible ongoing process involving the liquid.

The invention also concerns a system for distinguishing between particles with different magnetic properties, the system comprising

first detection means, such as a Hall sensor, for detection of the magnetic properties of particles and adapted to provide an output according to the magnetic properties of a particle comprised within a first measuring volume of the first detection means,

a population of particles made at least partly from a material with magnetic properties that are detectable by the first detection means, the population of particles comprising at least two subpopulations of particles, each subpopulation of particles having different magnetic properties so that said output from the first detection means may provide a significant indication of which subpopulation a detected particle is a member of,

a liquid in which the particles are contained when the magnetic properties of the particles are being detected by the first detection means, and

a member with a flow channel defined therein for leading the liquid with the particles contained therein through the first measuring volume, the flow of the liquid being controlled in such a way that one particle at a time passes the first measuring volume.

The particles comprised in the system for distinguishing between particles with different magnetic properties may have surface properties suitable for immobilising at least one reagent thereon and have reagents immobilised on a substantial number of the particles within each subpopulation so that each subpopulation has a specific reagent assign to it and at least two of the subpopulations have different specific reagents assigned to them, so as to enable performance of an analysis with at least two different specific reagents of the content of a liquid sample.

The system for distinguishing between particles with different magnetic properties may preferably comprise second detection means for detecting properties of reagents immobilised on particles comprised within a second measuring volume, for determining whether said properties have changed during an interaction between the content of the liquid sample and the particles, so as to perform an analysis of the content of the liquid sample.Preferably, the second measuring

volume is positioned and the flow of the liquid with the particles contained therein is controlled in such a way that particles passing the first measuring volume one at a time also passes the second measuring volume one at a time.

For controlling the flow in such a way that particles pass the first and optionally the second measuring volume one particle at a time, the flow channel defined in the member may optionally comprise means for entering at least one buffer-liquid into the flow channel parallel to the flow of the liquid with the particles contained therein.

<u>Brief description of figures</u>

Fig. 1 shows the forces acting on particles subjected to a magnetic field,

Fig. 2 shows a chip with an H-shaped channel therein,

Fig. 3 shows the H-shaped channel with adjacent magnets,

Fig. 4 is a diagram of an experimental setup including the chip with the H-shaped channel,

Fig. 5 and Fig. 6 A-D shows different stages of one experiment performed using the chip with the H-shaped channel,

Fig. 7, Fig. 8 and Fig. 9 shows expected results and actual results from the experiment performed using the chip with the H-shaped channel,

Fig. 10a illustrates competitive affinity assays,

Fig. 10b illustrates non-competitive affinity assays,

Fig. 11 shows the chemical principle of the IgG-assay,

Fig. 12a shows a linear compartment arrangement,

Fig. 12b shows five linear compartment arrangements placed in parallel,

Fig. 13 shows a compartment arrangement including a bend,

Fig. 14 shows a linear compartment arrangement comprising a number of magnets for letting the particles describe a zigzag path,

Fig. 15 shows an embodiment of a system adapted for performing light induced DNA synthesis,

Fig. 16 describes a process for synthesising oligomers of DNA,

Fig. 17 shows a setup for measuring the MRM-content in particles,

Fig. 18 shows a device for magnetic sampling and illustrates the method.

Detailed description of figures

Forces on particles

Fig. 1 shows the forces that act on magnetic particles 1 in a liquid system. F_m is the magnetic forces between the particles and the magnet 2, F_f is the friction force from the liquid acting on the moving particles 1, F_g is the gravitational force on the particles 1 and F_{ip} is the magnetic attraction between particles 1. The particles 1 are assumed to be out of contact with the walls that confine the liquid system. However, this assumption may not be valid for all cases that may be considered in connection with the invention.

Horizontal movement

The movement of a particle 1 towards the magnet 2 due to attraction between the magnet 2 and the particle 1 is called horizontal movement. The two forces involved in that movement are the magnetic force F_m (between the magnet 2 and the particle 1) and the friction force F_f (friction between the particle 1 and liquid).

The equation that governs the magnetic force on a single particle 1 is given as

$$F_m = \frac{1}{2} \cdot N_{fer} \cdot \frac{V_{fer} \cdot \Delta \chi_{fer}}{\mu_0} \cdot \nabla B^2$$

where N_{fer} is the number of magnetite pieces in a particle 1, V_{fer} is the mean volume of the magnetite pieces and $\Delta\chi_{fer}$ is the net magnetic susceptibility of magnetite (χ_{fer}-χ_{liquid}). Furthermore, μ_0 is the magnetic dipole moment of water and ∇B^2 is the gradient of the square of magnetic field of the magnet 2.

The friction force F_f acting on particles 1 where the Reynolds number for the movement of the particle 1 relatively to the surrounding fluid is less than 0.2, may be found from Stokes formula which gives the viscous drag force or friction force of a spherical particle 1 in a liquid as

$$F_f = 6 \cdot \pi \cdot R \cdot \eta \cdot \Delta v$$

where R is the radius of the particle 1, η is the viscosity given in Poise and Δv is particles speed relative to the flowing liquid.

The particles 1 may for most relevant situations be assumed to be in a quasi steady state so that

$$F_m = F_f$$

By substituting F_m and F_f with their equivalent expressions the following equation is achieved

$$\frac{1}{2} \cdot N_{fer} \cdot \frac{V_{fer} \cdot \Delta\chi_{fer}}{\mu_0} \cdot \nabla B^2 = 6 \cdot \pi \cdot R \cdot \eta \cdot \Delta v$$

from which the velocity of the particle 1 relatively to the fluid easily is isolated

$$\Delta v = \frac{1}{12} \cdot N_{fer} \cdot \frac{V_{fer} \cdot \Delta\chi_{fer}}{\mu_0 \cdot \pi \cdot R \cdot \eta} \cdot \nabla B^2$$

Parasitic movements

Movements of the particles 1 that are unwanted for the analytical purpose are called parasitic movements. Examples of these are gravitational settling of the particles 1, magnetic attraction between particles 1 and convection in the liquid, although these movements are wanted in some situations, e.g. where particles are moved by the gravitational force. Only gravitational settling is dealt with in the following.

Two forces are involved in gravitational settling. One is the force of the gravity acting on a particle 1 suspended in a liquid and the other is the friction between the particle 1 and the liquid.

According to Archimedes' equation, the net gravitational force acting on a particle 1 suspended in liquid is

$$F_g = (\rho_p - \rho) \cdot V_p \cdot g$$

where ρ_p and ρ are the densities of the particle 1 and the liquid, respectively, V_p is the volume of the particle 1 and g is gravitational acceleration.

The steady-state velocity of settling particles 1 can be calculated from the equality

$$F_g = F_f$$

When the forces are replaced by their formulas this gives

$$\Delta v = \frac{6 \cdot \pi \cdot R \cdot \mu}{(\rho_p - \rho) \cdot V_p \cdot g}$$

As the volume of a spherical particle 1 is given as $V_p = 4/3*R^3$, the settling velocity can be expressed as

$$\Delta v = \frac{(\rho_p - \rho) \cdot \frac{9}{2} \cdot R^2 \cdot g}{\pi \cdot \eta}$$

A first example of settling is given for Dynal M-280 particles 1 in a phosphate buffer. The particles 1 of a diameter of 2.8 μm and a density of 1300 kg/m3 are suspended in water with a density of 1000 kg/m3 and viscosity of 1 cP (10-3 kg/(m-1s-1)). This gives a settling velocity of

$$\Delta v = 8.3 \cdot 10^{-6} m/s$$

A second example of settling is given for Dynal M-280 particles 1 in a sucrose buffer with a density of 1240 kg/m3 and a viscosity of around 30 cP. The settling velocity is in this case found to be

$$\Delta v = 0.055 \cdot 10^{-6} m/s$$

The gravitational settling may be reduced by counteracting the gravitational force with e.g. a magnet placed above a channel in which magnetic particles are situated.

A system that has been used for the initial tests of the magnetic particle handling is shown in Figs. 2-9 together with the results of the test. Fig. 2 shows the flow chip 3 that was used, comprising a top part 4 (8*40*15 mm) and a lid 5 (2*40*15 mm) both made from PMMA. An H-shaped channel 6 was milled in the surface of the top part 4. The channel 6 has a depth of 400 μm and a width of 400 μm. The H-shaped channel 6 is 30 mm long, and the interconnection line 7 is 5 mm wide. 1.4 mm diameter holes 8 were drilled at each of the four ends of the H. A piece of tubing, 1/16 inch outer diameter and 0.8 mm inner diameter, was inserted in each hole 8 so as to form a liquid interface from the exterior to the channel 6. The milled surface of the top part 4 was wetted with acetone and pressed together with the lid 5. The combined of top part 4 and lid 5 is shown in Fig. 2 on the right. A fluorescence microscope 9 equipped with a photo multiplier tube for light detection was provided for monitoring of the interior of the channel.

Fig.3 shows the positioning of the magnets relative to the liquid filled channels 6. The primary magnets 10 are optionally placed in such a way that they may provide a force by which the particles 1 may be moved back and forth in the interconnection channel 7. Preferably, electromagnets 10 are used, comprising an iron core with length 80 mm and diameter 6 mm and about 400 windings of a 0.5 mm outer diameter copper wire. At the end surface of the electromagnets 10 a magnetic flux density of at least 0.22 Tesla (at a voltage of 3.1V and a current of 3.3A) may be achieved. The primary magnets 10 are placed as close as possible to the interconnection channel 7 of the flow chip 3 to optimize the effect from the magnets 10 on the particles 1.

A secondary magnet 11 is provided as a counter measure to prevent the particles 1 from settling and is a SamariumCobalt permanent magnet 11 (10*6*30 mm) from Goudsmit, The Netherlands. The force from the secondary magnet 11 on the 1 particles is in a direction opposite to the force of gravity.

The magnetic flux density is 0.5 Tesla at the surface of the permanent magnet 11. The secondary magnet 11 is placed approximately 25 mm above the H-shaped channel 6 to achieve the reduction in settling of particles 1.

To automate the filling of compartments and the assay procedure, a computer controlled flow injection system was set up. Fig. 4 shows a schematic view of the system including the flow chip 3. The full lines 12 indicate PFTE teflon tubing of outer diameter of 1/16 inch and inner diameter of 0.8 mm for providing liquid connection between the different components, together with suitable fittings. The dotted lines 13 indicate data communication means for providing data communication between the Personal Computer 14 and various components. Two step motor driven syringe pumps 15, 16 were used for driving liquid through the H-shaped channel 6. Three three-way valves 17 are used for precise liquid control, such as for filling one of the pumps 15 with buffer from the buffer reservoir 18, for aspiration of particle suspension from the suspension vial 19 and for addressing the individual outlets of the flow chip 3.

The magnets 10 (only one is shown) and the detection system 9 are also indicated in Fig. 4. All the active components are linked to a Personal Computer 14 that is equipped with a multifunction card. The multifunction card and the control software is from National Instruments, USA.

The particles used in the present experiment are from Dynal AS, Norway. The particles have a density around 1.3 g/mL and are superparamagnetic. They have streptavidin immobilised on their surface which allows for coupling with biotin-bound reagents and aminoreactive dyes.

The particles were labelled with fluorescein isothiocyanate (FITC) according the following prescription: 10 mg FITC was brought into solution in 1 mL dimethylsulfoxide. 1000 μL magnetic particle suspension was transferred to a dark duran

glass flask and added 10 mL pH 9.5 carbonate buffer (0.1 M) and 500 μL FITC-solution. The mixture was shaken well and allowed to react for 12 hours at room temperature. Unbound FITC/fluorescein was removed from the labelled particles by successively washing and separating the particles magnetically from the washing liquid until no more background fluorescence from the liquid was detected. A pH 7.4 phosphate (0.1 M) buffered saline solution was employed both for the washing process and as an inert buffer for the characterisation. For the final experiments the effective dilution of the manufactures stock solution was 1/330 giving 0.03 mg particles/mL.

A more detailed picture of the compartment complex used for the characterisation experiments is shown in Fig. 5. The H-shaped channel 6 comprises the compartment for particle supply 20, the compartment for detection 21 of properties of the particles and the compartment for sample interaction 7. The two primary magnets 22, 23 are placed outside the H-shaped channel 6 in such a way that the steepest gradient of the magnetic field goes through the compartment for sample interaction 7. The compartment for particle supply 20 can be replenished by sending in a suspension of particles at the inlet 24 and let the used suspension leave the compartment at the outlet 25. The inlets 24, 26 and outlets 25, 27 can be addressed individually through the use of external valves (not shown in the figure), which allows for flexible filling of the compartments.

The particles 1 in suspension have higher density than the liquid in which they are suspended but the buoyancy force is partly cancelled out by the secondary magnet 11. The compartment for sample interaction 7 is in the characterisation experiments filled with a buffer that does not alter the fluorescent properties of the beads.

The detection window 28 in the compartment for detection 21 is represented as a black rectangular box in Fig. 5. The

actual shape of the detection window 28 is defined by a pinhole in front of the photomultiplier of the fluorescence microscope.

The procedure of running one experiment is described in Fig. 6 A-D). The compartments are first filled with the liquid that they are supposed to contain. The detection compartment 21 and compartment for sample interaction 7 are filled with phosphate buffer and subsequently the compartment for particle supply 20 is filled with a suspension of particles 1. Fig. 6 A) illustrates the condition where all the compartments are replenished.

The first magnetic action is to align the particles 1 in the compartment for particle supply 20. This is accomplished by turning on the primary magnet 22 closest to the supply compartment 20. Within less than 15 seconds the particles 1 are aligned as shown in Fig. 6 B). The next step is to turn off the aligning magnet 22 and to turn on the primary magnet 23 that will pull the particles 1 through to compartment for sample interaction 7, this step is called the launch. Bigger particles and aggregates of particles 29 will move faster that single and smaller particles 1 which is also shown in Fig. 6 D). When the particles 1 have passed the detection window 28 and the measurements have been made, the compartments 7, 20, 21 may be filled up again and a new analysis or synthesis can be performed.

The expected result of the experiment is shown in Fig. 7. The arrival of a fluorescent particle 1 to the detection window 28 and the measuring volume of the detector 9 results in a peak. Both the time of flight, which is the time from launch to the arrival at the detector, and the peak width are important fingerprints of the identity of the particle 1. The first particles 1 to cross the detection window will be the biggest since bigger particles 1 move faster than smaller ones. The bigger particles 1 will move faster through the

detection area than smaller ones due to their higher speed, thus making narrower peaks.

In the experiment with inert buffer in the compartment for sample interaction 7, the measured response from a single particle 1 will be independent of its time of flight. For the intended use, an interaction may take place between molecules in the sample placed in the compartment for sample interaction 7 and one or more reagents immobilised on the particles 1. In the case of slow kinetics of these interactions, the time of flight will be highly correlated with the change in response of the particles 1. If each peak is plotted as peak height versus the time of flight, a pattern similar to the one shown in Fig. 8 would be expected. The measured responses that fall within the marked diagonal area are considered credible as they are the product of a controlled magnetic manipulation. Measurements that fall outside the marked area are called forbidden as the response have reached the detector 7 in a uncontrolled manner. The credible peaks should be given the most weight in the data analysis. Only peaks within the solid black parts of the marked diagonal would be expected if an experiment where only single particles and aggregates of two particles are present is considered.

A plot of measured responses of an actual characterisation experiment is shown in Fig. 9. The plot in Fig. 9 reveals a clear tendency in the measurements towards that the narrowest and highest peaks, which are significant for the aggregates, are detected first and the broader and lower ones, indicating single particles, are detected later.

<u>Affinity assays - an immunoassay</u>

The affinity assay is one of the working horses of analytical biochemistry. The affinity assay uses pairs of molecules that bind together with high strength, affinity. Such pairs are antibody-antigen, avidin-biotin, chelate-metal, enzyme-

inhibitor, DNA-DNA, RNA-RNA, etc. The reagent part of the affinity pair is often immobilised on a solid phase and the analyte part is sought in a liquid sample.

The solid phase is in the present case the particles 1, e.g. susceptible to magnetic fields, and the analytes are captured by the reagent immobilised on the particles by letting the magnetic particles move through the sample. The affinity assays are typically performed in one of two modes named competitive and non-competitive assays. The chemical principle of each mode will briefly be explained in the following together with a description of two embodiments of the invention.

Competitive affinity assays

Labelled and unlabelled analyte are competing about affinity ligands immobilised on a solid phase in the competitive affinity assay of which the principle is shown in Fig. 10a. The solid phase 30 has been coated with the affinity ligand 31 and is surrounded by a liquid media containing unlabelled analyte 32, which is native to the sample and is present in unknown amounts, and labelled analyte 33 that is added in known amounts. An important feature is that the amount of labelled analyte 33 should be in excess compared to the amount of affinity ligand 31 immobilised on the solid phase 30. The solid phase 30 is washed with an in washing buffer after a certain reaction time and the remaining response from the labels on solid phase is measured with detection means, such as an optical fluorescence sensor. The ratio of labelled 33 and unlabelled analyte 32 on the solid phase 30 should the same as the ratio in the sample when the labelled analyte is added, assuming similar reaction kinetics for the labelled and unlabelled analyte. The amount of analyte 32 native to the sample can be calculated since the amount of labelled analyte 33 is known.

The labels used for labelling an analyte 33 should be easy to detect with appropriate detection means and be of high sensitivity. Fluorescing probes like fluorescein, rhodamine and Texas Red are often used as labels. These probes exist in derivatives that are easily coupled to common analytes. Another type of label that is often used is enzymes. The enzymes are catalytic molecules and the presence and concentration is detected by the amount of signal yielding reaction they cause, and enzyme labels are rather sensitive due to their signal amplifying abilities.

Non-competitive affinity assays

Two types of affinity ligands are employed in the non-competitive affinity assay as illustrated in Fig. 10b. The primary ligand 34 is immobilised on the solid phase 30 as was the case for the competitive assay. An amount of analyte 32 from the sample and secondary, labelled affinity ligands 35 that also bind to the analyte 32 are present in the surrounding liquid. The amounts of primary and secondary antibodies have to be in excess compared to the amount of analyte 32.

The analytes 32 will be bound to the solid phase 30 via the primary affinity ligand 34 and each bound analyte 32 will be attached a labelled secondary affinity ligand 35. The solid phase 30 is after some reaction time washed with a suitable buffer and the response from the solid phase 30 is measured with detection means, such as an optical fluorescence sensor. The measured response is proportional to the amount of analyte 32 captured by the solid phase 30 whereas the response from the competitive assay was proportional to the ratio between labelled 33 and unlabelled 32 analyte. This fact renders the non-competitive assay more sensitive than the competitive one.

One special application of the non-competitive affinity assay is making the diagnosis of viral infection (HIV, Epstein-Barr

virus, influenza, etc.). To indicate the presence of the virus in a person, a blood sample is taken and analyzed for antibodies (immuno globulin G, IgG) against the specific virus. Fig. 11 shows the chemical principle of the IgG-assay. Particles 1, e.g. susceptible to a magnetic field, have been coated with surface groups 36 from the given virus. The IgG's 37 from the blood sample - specific to the virus - will normally bind to the viral surface groups 36 and therefore attach themselves to the particles 1. To make the presence of IgG's 37 detectable, secondary affinity reagents 35 may be employed. The could be protein A (from Staphylococcus aureus) labelled with a fluorescent label which could be fluorescein-isothiocyanate (FITC). When all the component have had time to interact, the particles 1 have become fluorescent due to the protein A-FITC that have bound to the virus specific IgG's 37 that have bound to the viral surface groups 36 on the particles 1. The fluorescence from the particles 1 is then detected.

Affinity assay embodiment

The procedures for making an affinity assay may be implemented in several ways employing magnetic particle manipulation. Two preferred embodiments will be described in the following.

The first system shown in Fig. 12a is based on a linear compartment arrangement, where the compartment for particle storage 38 is followed by the compartment for sample interaction 39 and finally the compartment for secondary reactions and/or for washing 40 comprising the measurement area 41 for detection of properties of the particles 1.

The secondary reaction and/or washing compartment 40 may when used for competitive assays analysis be filled with a liquid for washing or a liquid containing labelled analyte. When used for a non-competitive assay analysis, the secondary

reaction compartment 40 may be filled with a liquid containing the secondary, labelled affinity ligand.

An electromagnet 42 is placed at the right end of the linear complex of compartments to move the magnetic particles 1 coated with the primary affinity ligand through the compartments.

The complex of compartments sketched in Fig. 12a may be used as a disposable one-shot system, with magnetic particles 1 and secondary chemicals pre-filled into the compartments. The electromagnet 42 and the detection system are physically separated from the disposable compartment complex and can be used many times. The only liquid that should be injected during operation of the complex of compartments for performing a test is the sample. Therefore, the only openings for passing liquid between the complex of compartments and the exterior that are open after the pre-filling of the compartments are the ones in the compartment for sample interaction 39 which has a sample inlet 43 and a sample outlet 44.

The complex of compartments may be made from a polymer e.g. by injection moulding or conventional milling. The dimensions of the shown embodiment are a compartment cross section of 400 μm times 400 μm, the length of the particle storage compartment 38 is 500 μm, the length of the compartment for sample interaction 39 is 5000 μm and the length of the washing/detection compartment 40 is 3000 μm.

The linear compartment complex as the one shown in Fig. 12a has the advantage that a number of complexes may easily be placed in parallel as shown in Fig. 12b. Here, five linear compartment complexes are arranged in parallel with respect too the direction of movement of the particles 1. The sample interaction compartments 39 of the compartment complexes are connected in series and are supplied with sample from a common sample inlet 43. Each linear compartment complex is

adapted for measuring with a specific analyte or to make more tests for the same analyte in order to enhance precision and to provide a validation for the results of the measurements.

If the involved affinity ligands are antibodies one could use magnetic particles 1 coated with protein A or G (which bind most IgG antibodies in a favourable manner), streptavidin or biotin (e.g. streptavidin on the particles and biotin coupled to the antibodies) or reactive leaving groups as tresyl chloride or tosyl chloride. Such particles can be purchased from Bangs Laboratories (US) and Dynal (Norway).

Compartment complexes with the same function (e.g. affinity assays) may be arranged together in many ways. Fig. 13 shows a sketch of an alternative arrangement for the affinity assay. If longer compartment lengths are needed without making the electromagnets stronger a bend between the sample interaction compartment 39 and the compartment for secondary reactions and/or for washing 40 may be made. The complex of compartments comprises in this case two magnets 45, 46, one magnet 45 for moving the particles 1 from the particle storage compartment 38 and through the compartment for sample interaction 39, and one magnet 46 for moving the particles 1 through the compartment for secondary reaction 40 and into the measurement area 41. The effect of increasing the length of the sample interaction compartment 39 is that the particles 1 will interact with more sample when passing through the compartment 39 with means that the particle 1 have opportunity to interact with more analyte and the analysis thus becomes more sensitive.

There are alternative ways of generating the magnetic gradients. As shown in Fig. 14 several magnets 47, preferably electromagnets, AC or DC, may be positioned along both sides of the linear compartment complex. Each magnet 47 may be actuated individually and if the actuation is timed well the magnetic particles 1 will move following a zigzag path through the complex. In this way, the particles 1 are allowed

to interact with more of the contents of the liquids in the compartment system than if the particles 1 followed a straight path through the complex, thus enhancing the sensitivity of the analysis.

DNA synthesis

The synthesis of short strands of DNA is of importance when dealing with genetic analysis. Typical applications include screening for pathogenic micro organisms and diagnosing genetic disorder. Short nucleotide probes can also be used as synthetic antibodies, which is very interesting since DNA is more stabile than protein. This means that short nucleotide probes may act as generic affinity ligands.

Light induced dna synthesis

DNA is a polymer consisting of 4 different monomer units: adenosine (A), thymine (T), cytosine (C) and guanine (G). The monomer units can be randomly combined giving the single DNA strand different geometric shapes and genetic coding.
The process of synthesising oligomers of DNA by the means of controlled light activation is well established (reference: 'The efficiency of Light -Directed Synthesis of DNA-arrays on Glass Substrates', McGall et al, J. Am. Chem. Soc., 119 (22), 1997, p. 5081-5090) and even used in some commercial systems (Affymetrix, USA).

A process for synthesising oligomers of DNA is described in Fig. 16. The magnetic particles 1 are coated with a light sensitive molecule 48, as shown in Fig. 16 A., with an oxygen bridge that dissociates upon exposure to light of a certain wavelength as shown in Fig. 16 B-C. When the particles 1 are moved into a compartment with preactivated nucleotides, 'X-■ 49, the nucleotides 49 will bind to the activated sites 48 of the particle 1 and retain the ability to be activated itself as shown in Fig. 16 D-E. The "X" of the nucleotides 49

symbolises that the compartment may have been filled with any of the four nucleotides. By repeating the steps in Fig. 16 A-E four time and switching between compartments of thymine (T), cytosine (C), adenosine (A), and thymine (T), one could obtain the sequence: T-C-A-T as shown in Fig. 16 F.

An embodiment according to the invention of a system adapted for performing light induced DNA synthesis is presented in Fig. 15. Four compartments 50-53 for nucleotide-particle interaction (CNPI) are each filled with a pure solution of one of the four nucleotides so that each nucleotide is represented. The four CNPI's are combined with washing compartments 54 and an activation compartment 55 in such a way that a magnetic particle 1 can travel between the compartments 50-55 in any order that should be desired. Four electromagnets 56 are positioned so that they may move the particles between the compartments 50-53 containing the CNPI's by their magnetic fields. Additionally, magnets could be included to reduce settling effects from the gravitational field.

The CNPI's are sketched with inlets 57 and outlets 58 in order to allow for replenishing of the liquid in the compartments 50-55. The complex could also be made for one-shot purposes without allowing replenishment. The light activation compartment 55 should allow light of the wavelength of activation to reach the interior of the compartment 55, e.g. by having a top or bottom wall that is transparent to the wavelength of activation.

The true force of the method of magnetic particle 1 manipulation is unleashed when synthesis and analysis is combined in a kit. The synthesis of DNA affinity ligands may preferably be computer controlled, thus the result of the synthesis may be determined by the software/user and not the reagents initially immobilised on the beads. Therefore, the user may choose to analyse for any analyte that can be bound by a DNA-oligomer using the same system, since the affinity

reagents are generated just before the analysis. This arrangement allows for great flexibility and applicability of a single system.

Magnetic barcoding

The principle of magnetic bar coding is to identify individual particles as belonging to a specific subpopulation of particles among a number of subpopulations by detecting the magnetic properties of the particles 1, e.g. the amount of magnetically responsive material (MRM) in the particles 1. The particle 1 will, depending on its MRM loading, create a distortion of an external magnetic field and this distortion may be measured with a Hall-sensor that may be either integrated in the chip with the flow channel or be external.

The particles 1 are divided in subpopulations based on the MRM-loading (or similar loading per volume and different size). The differences in MRM loading (or size) between the subpopulations should be big enough to distinguish between them with the Hall-sensor. It is possible to achieve a high number of subpopulations if desired, at least 50-100 and up to 200 groups with some effort. The two mayor applications of this aspect of the invention are particle based multi analyte assays and test of small combinatorial libraries.

Multi analyte assays

For the multi analyte assay one needs a population of particles 1 consisting of many subpopulations. Each subpopulation of particles is to be coated with a certain reagent. The subpopulations are mixed into one population and exposed to the sample. If analytes are present the in sample the magnetic particles with the corresponding reagent will change their response. By looking at the particles separately both their magnetic content and analytical response can be measured.

A possible setup for measuring the MRM-content in the particles is shown in Fig. 17. A suspension of particles 1 that have been incubated with a sample is hydrodynamically focused by sending it through a center flow channel 59 and sandwich it between two streams of inert buffer 60. In this way, the particles 1 are tightly positioned in the measurement channel 61 so that the particles 1 passes a cross-section of the measurement channel 61 one at a time. A magnet 62, e.g. a permanent magnet or an electro magnet, is placed so that its field penetrates the measurement channel 61 and a Hall-sensor 63 is positioned on the opposite side of the channel 61. The Hall-sensor 63 measures the flux density of the magnetic field and detects changes in the field caused by the magnetic particles 1. Besides the Hall-sensor 63 an optical measuring window 64 is placed in the channel to make it possible to correlate the magnetic properties measured by the Hall-sensor 63 to the optical measured properties for each specific particle 1. For example, fluorescence may be measured from the particles through the optical measuring window 64 with a fluorescence detector, such as a photomultiplier or a CCD array arranged with a suitable objective.

Optionally, two pieces 65 of soft magnetic material may be inserted on each side of the measurement channel 61 to focus the magnetic field so that the measurement volume of the Hall-sensor 63 is decreased and the spatial resolution thereby is increased.

<u>Magnetic sampling</u>

Sampling in analytical chemistry covers the operation of taking out a representative subpart of the entire liquid (or solid or gas) that is to be analyzed. Representative sampling is a far-from-trivial problem that is often not a part of automated analysis systems. A common approach is to take out a liquid sample from one point and assume that the composition of the remaining liquid is similar to the sample.

The novelty of the proposed method according to the invention, magnetic sampling, is to probe the entire liquid with magnetic particles coated with analyte specific reagents, instead of taking out and analysing a small subpart of the sample, so-called subpart sampling. Reagent coated magnetic particles 1 are suspended in the medium that is to be analyzed, see Fig. 18, where the liquid in the shown example is contained in a stirred liquid container 66. The container 66 could for example be a fermentation tank for biotechnological production, a waste-water treatment plant or lake. The magnetic particles are, when a magnet 67 is activated, moved from the liquid to be analyzed to the analysis system through a liquid connection between the container 66 and the analysis system.

Conventional techniques/methods known in the art of chemical analysis (e.g. flow injection with fluorometry or absorptivity) may be used in the analysis system.

An advantage of the magnetic sampling compared to subpart sampling is that the probe particles 1 can preconcentrate the analyte of interest on their surface. Therefore, the surface concentration of analyte on the particles 1 will be magnitudes higher than the concentration in the entire liquid to be analyzed, and the upcoming analysis will have an increased sensitivity to the analyte.

Furthermore, magnetic sampling is more robust than subpart sampling. Sucking out a heterogeneous liquid sample may lead to clogging in the analysis flow system followed by malfunction. For the analysis magnetic sampling only takes in the well-defined probe particles. Therefore the risk of clogging and system contamination is significantly reduced.

When no more analysis are required, the magnetic probe particles are easily removed from the main liquid with a magnetic field.

Another new feature is sequentially to collect the particles 1 during the time of monitoring. This is done by applying a magnetic field by activating the magnet 67 and then analyse the particles 1 as soon as they enter the analysis system. This will give a timely resolution of the measured parameters in the liquid to be analyzed and is usable for chemical process control and environmental monitoring.

A third new feature is shown in Fig. 19 a-c. The reagent coated magnetic particles 1 are not suspended in the unknown liquid, but are moved to the interface 68 between the analysis systems 69 and the liquid to be analyzed 70 as shown in Fig. 19a and held there as shown in Fig. 19 b, where both the movement and the holding is performed with magnetic forces. At the interface 68, the reagent coated magnetic particles 1 will interact with the liquid to be analyzed 70 for a controlled time period and will afterwards be moved back into the analysis system to complete the analysis as shown in Fig. 19c.

CLAIMS

1. A method of moving a particle with at least one reagent immobilised thereon into a liquid sample that is contained in a compartment system comprising a first compartment that is interconnected with a second compartment, each of which having an opening defined therein, the method comprising the steps of

entering at least one particle with at least one reagent immobilised thereon into the first compartment,

entering the liquid sample into the second compartment,

subjecting the compartment system to a field to which the at least one particle is susceptible whereby the at least one particle is moved into the second compartment by a force exerted on the at least one particle by the field so that the reagent on the at least one particle may interact with the content of the liquid sample contained in the second compartment in such a way that substantially no convection of the liquids in the compartment system takes place during said movement and said interaction.

2. A method according to claim 1, wherein the step of subjecting the compartment system to a field comprises the step of positioning field generating means for generation of a field that is subjected to at least a part of the compartment system at the compartment system.

3. A method according to claim 1 or 2, wherein the step of subjecting the compartment system to a field comprises the step of generating a magnetic field.

4. A method according to claim 2, wherein the step of positioning field generating means at the compartment system comprises positioning an electro magnet at the compartment system and the step of subjecting the compartment system to a

field comprises activating the electro magnet with an electric current.

5. A method according to claim 2, wherein the step of positioning field generating means at the compartment system comprises positioning two electrodes in electrical contact with the liquid in the compartment system and the step of subjecting the compartment system to a field comprises the step of supplying an electric potential between the two electrodes so that the at least one particle is moved by electrophoresis.

6. A method according to claim 2, wherein the step of positioning field generating means at the compartment system comprises positioning two electrodes at the compartment system in such a way that they are not in electrical contact with the liquid in the compartment system and the step of subjecting the compartment system to a field comprises the step of supplying an electric potential between the two electrodes so that the at least one particle is moved by dielectrophoresis.

7. A method according to claim 1, wherein the step of subjecting the compartment system to a field comprises the step of centrifugation of the compartment system.

8. A method according to claim 1, wherein the step of subjecting the compartment system to a field comprises the step of subjecting the compartment system to the field of gravitation.

9. A method according to any of claims 1-8, further comprising the step of

monitoring properties of the at least one particle during the interaction.

10. A method according to any of claims 1-9, further comprising the step of

monitoring properties of the at least one particle after the interaction.

11. A method according to claim 10, wherein the compartment system further comprises a third compartment that is interconnected with the second compartment, the method further comprising the step of

moving the at least one particle by means of the field into the third compartment so as to perform the monitoring of the properties of the at least one particle when the at least one particle is situated in the third compartment.

12. A method according to claim 10 or 11, wherein the compartment system further comprises a secondary interaction-compartment that is interconnected with the second compartment, that prior to the step of monitoring further comprises the steps of

moving the at least one particle by means of the field into the secondary interaction-compartment of the system, and

allowing the at least one particle to interact with a liquid contained in the secondary interaction-compartment so as to make the result of the interaction between the reagents and the content of the liquid sample detectable by the detection means.

13. A method according to any of the preceding claims, wherein the compartment system further comprises a washing-compartment that is interconnected with any of the other compartments and comprising the steps of

moving the at least one particle into the washing-compartment of the compartment system by means of the field, and

allowing the at least one particle to interact with a liquid contained in the washing-compartment so as to remove unwanted material from the at least one particle.

14. A method according to any of the preceding claims, wherein one of the compartments is adapted for letting electromagnetic radiation of certain wavelengths reach the liquid contained in said compartment, the method further comprises the step of

subjecting the at least one particle to electromagnetic radiation of a wavelength suitable for causing a process of photoactivation.

15. A method according to any of the preceding claims, wherein the at least one particle is of a mean diameter of 1-200 micro meter.

16. A method according to any of the preceding claims, wherein the cross-sectional dimensions of said compartments are within 100-1000 micro meter.

17. A device comprising a system of compartments in which the content of a liquid sample may interact with at least one reagent immobilised on at least one particle, the device comprises

at least one particle with surface properties suitable for immobilising at least one reagent thereon,

at least one reagent suitable for being immobilised on the surface of the at least one particle,

a first compartment for storage of the at least one particle,

a second compartment in which the liquid sample may interact with the reagent immobilised on the at least one particle, each of said first and second compartment having at least one

opening for passing liquids between the compartment and the exterior,

means for subjecting at least a part of the system to a field so as to move the at least one particle between the first and the second compartment, and

a passage defined between the first compartment and the second compartment so as to allow the at least one particle to be moved from one compartment into the other compartment through the passage.

18. A device according to claim 17, wherein the second compartment further comprises a second opening for passing liquids between the compartment and the exterior.

19. A device according to claim 17 or 18, wherein the compartment system comprises at least one field generating means adapted to apply a field to at least a part of the compartment system, and the at least one particle being at least partly made from a material susceptible to the generated field.

20. A device according to claim 19, wherein the generated field is a magnetic field.

21. A device according to claim 20, wherein the field generating means comprise at least one electro magnet.

22. A device according to claim 19, wherein the field generating means comprises two electrodes in electrical contact with the liquid in the compartment system so that the field generating means are activated by applying an electrical potential difference between the two electrodes and the at least one particle is moved by electrophoresis.

23. A device according to claim 19, wherein the field generating means comprises two electrodes which are not in

electrical contact with the liquid in the compartment system so that the field generating means are activated by applying an electrical potential difference between the two electrodes and the at least one particle is moved by dielectrophoresis.

24. A device according to claim 19, wherein the field is generated by centrifugation of the compartment system.

25. A device according to claim 17 or 18, wherein the field is a gravitational field.

26. A device according to any of claims 17-25 and comprising

detection means for detecting properties of the at least one reagent immobilised on the surface of the at least one particle.

27. A device according to claim 26 and comprising a third compartment for performing the detection of the properties of the at least one reagent immobilised on the surface of the at least one particle with the detection means, said third compartment comprising

an opening for passing liquids between the compartment and the exterior,

an area that is transparent to allow of optical access from the exterior to the interior of the compartment, and

a passage defined between the second compartment and the third compartment so as to allow particles to be moved between the second compartment and the third compartment, the means for subjecting at least a part of the system to a field being adapted for moving the at least one particle between the second compartment and the third compartment by said field.

28. A device according to any of claims 17-27 and comprising

at least one auxiliary compartment, said auxiliary compartment comprises an opening for passing liquids between the compartment and the exterior, and

a passage defined between the auxiliary compartment and one of the other compartments so as to allow particles to be moved between said compartment and the auxiliary compartment,
the means for subjecting at least a part of the system to a field being adapted for moving the at least one particle between the auxiliary compartment and said compartment by said field.

29. A device according to any of claims 19-28, wherein the generating means of the system are adapted for moving the at least one particle both back and forth between compartments between which a passage is defined.

30. A device according to any of claims 17-29, wherein one of the compartments is adapted for letting light of certain wavelengths reach the liquid contained in said compartment.

31. A device according to any of claims 17-30, wherein the at least one particle is of a mean diameter of 1-200 micro meter.

32. A device according to any of claims 17-31 wherein the cross-sectional dimensions of said compartments are within 100-1000 micro meter.

33. A device according to any of claims 17-32, wherein the system of compartments are manufactured from materials that are non-magnetic.

34. A device according to any of claims 17-33, wherein the system of compartments are manufactured from materials that are non-autofluorescent, such as Topas.

35. A device according to any of claims 17-34, wherein the interconnections between compartments are closed until they by activation are opened prior to the performance of a test so as to prevent the contents of the compartments to mix before a test.

36. A device according to claim 35, wherein the interconnections are closed with a material that is solid before activation, and the activation is performed by heating at least a part of the system of compartments until the closing material becomes liquid.

37. A device according to claim 35, wherein the activation of the interconnections is performed by physically aligning the compartments.

38. A method of analysing the content of a liquid contained in a container, the method comprising the steps of

(a) mixing particles with the liquid so as to obtain a substantially even distribution of the particles in at least a part of the liquid, the particles being at least partly made from a material susceptible to a field, such as a magnetic field or an electric field, and having at least one reagent immobilised on a plurality of the particles,

(b) allowing the reagent immobilised on the particles to interact with the content of the liquid,

(c) applying a field to which the particles are susceptible to at least a part of the container so as to move at least one of the particles through an opening of the container to extract the at least one particle from the container,

(d) moving the at least one particle through a liquid-filled passage to detection means for detecting

properties of the reagents on said at least one particle, and

(e) detecting properties of the reagent on said at least one extracted particle in order to determine whether these properties have changed due to the interaction, so as to perform an analysis of the liquid.

39. A method according to claim 38, wherein the steps (c) to (e) are repeated at least once after elapse of a predetermined time period so as to provide a monitoring of a possible ongoing process involving the liquid.

40. A system for distinguishing between particles with different magnetic properties, the system comprising

first detection means, such as a Hall sensor, for detection of the magnetic properties of particles and adapted to provide an output according to the magnetic properties of a particle comprised within a first measuring volume of the first detection means,

a population of particles made at least partly from a material with magnetic properties that are detectable by the first detection means, the population of particles comprising at least two subpopulations of particles, each subpopulation of particles having different magnetic properties so that said output from the first detection means may provide a significant indication of which subpopulation a detected particle is a member of,

a liquid in which the particles are contained when the magnetic properties of the particles are being detected by the first detection means, and

a member with a flow channel defined therein for leading the liquid with the particles contained therein through the first measuring volume, the flow of the liquid being controlled in

such a way that one particle at a time passes the first measuring volume.

41. A system according to claim 40, wherein the particles have surface properties suitable for immobilising at least one reagent thereon and reagents are immobilised on a substantial number of the particles within each subpopulation so that each subpopulation has a specific reagent assign to it and at least two of the subpopulations have different specific reagents assigned to them; so as to enable performance of an analysis with at least two different specific reagents of the content of a liquid sample.

42. A system according to claim 41 and comprising second detection means for detecting properties of reagents immobilised on particles comprised within a second measuring volume of the second detection means, for determining whether said properties have changed during an interaction between the content of the liquid sample and the particles, so as to perform an analysis of the content of the liquid sample.

43. A system according to claim 42, wherein the second measuring volume is positioned and the flow of the liquid with the particles contained therein is controlled in such a way that particles passing the first measuring volume one at a time also passes the second measuring volume one at a time.

44. A system according to any of claims 40-43, wherein the flow channel defined in the member comprises means for entering at least one buffer-liquid into the flow channel parallel to the flow of the liquid with the particles contained therein, for controlling the flow in such a way that particles pass the first and optionally the second measuring volume one particle at a time.

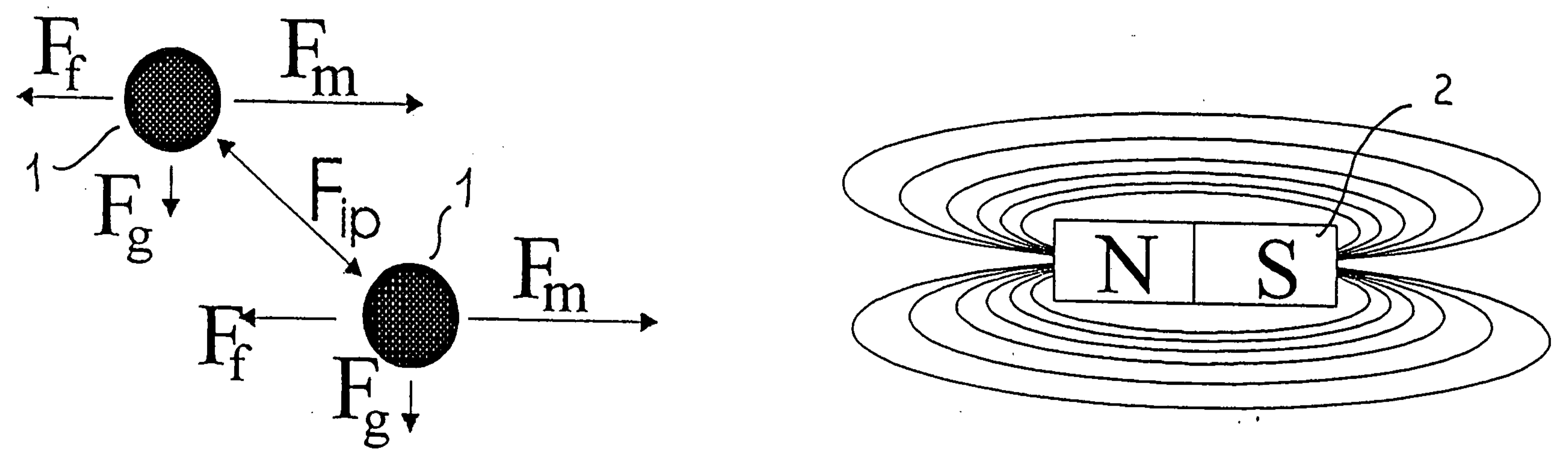


Fig. 1

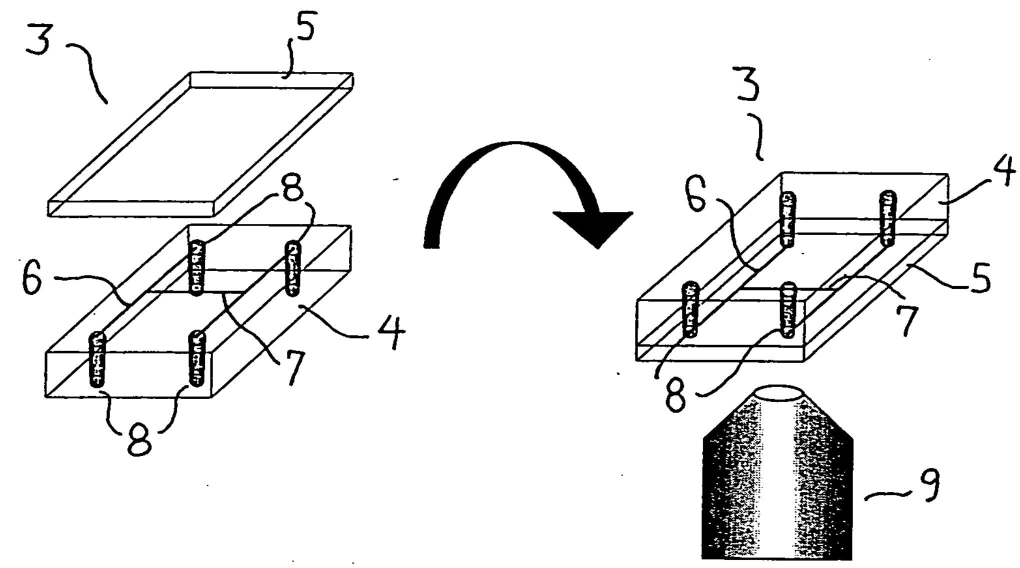


Fig. 2

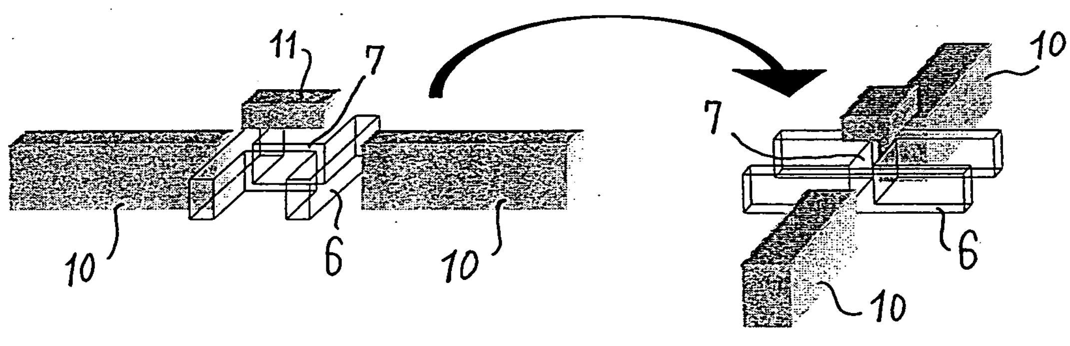


Fig. 3

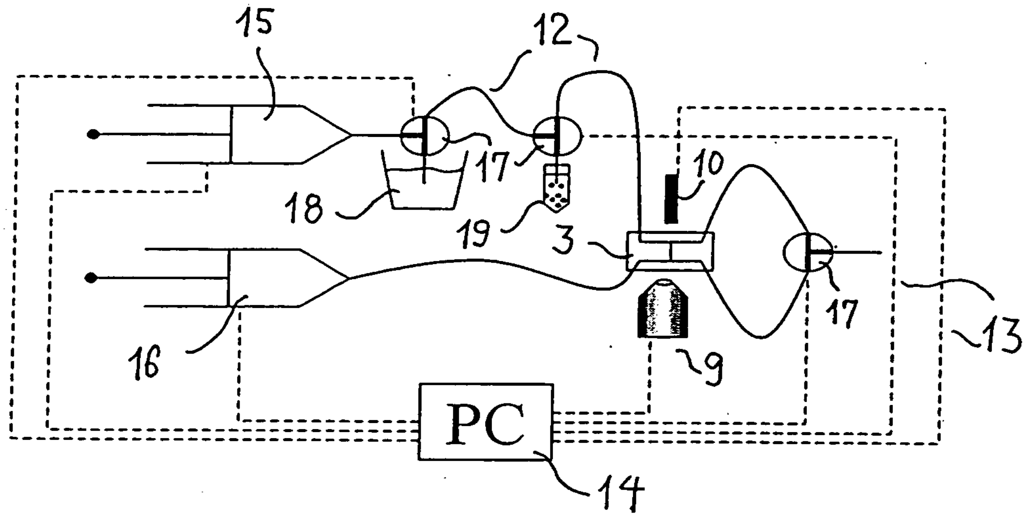


Fig. 4

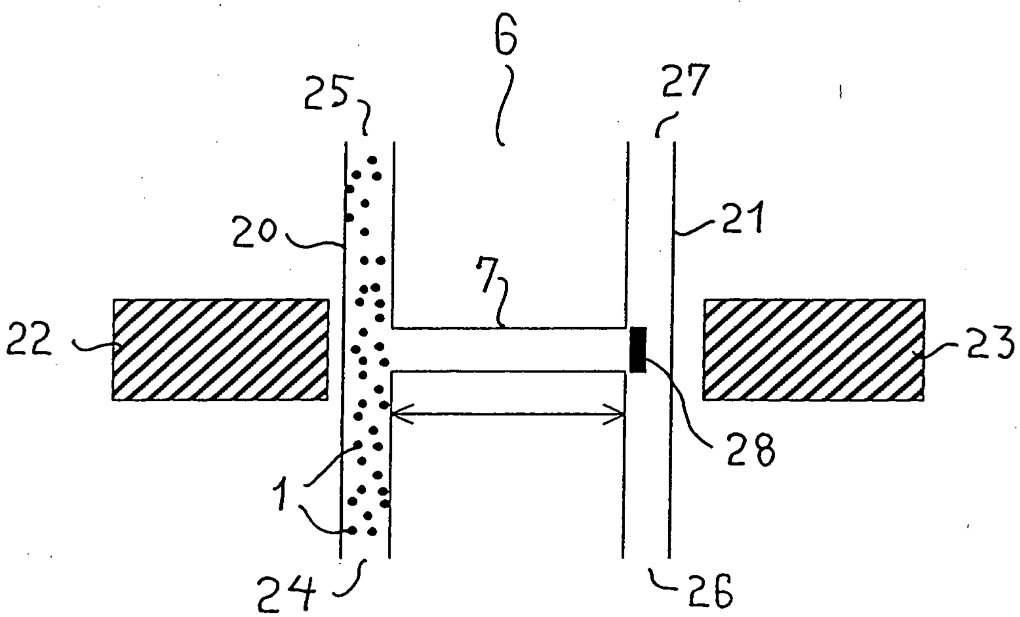


Fig. 5

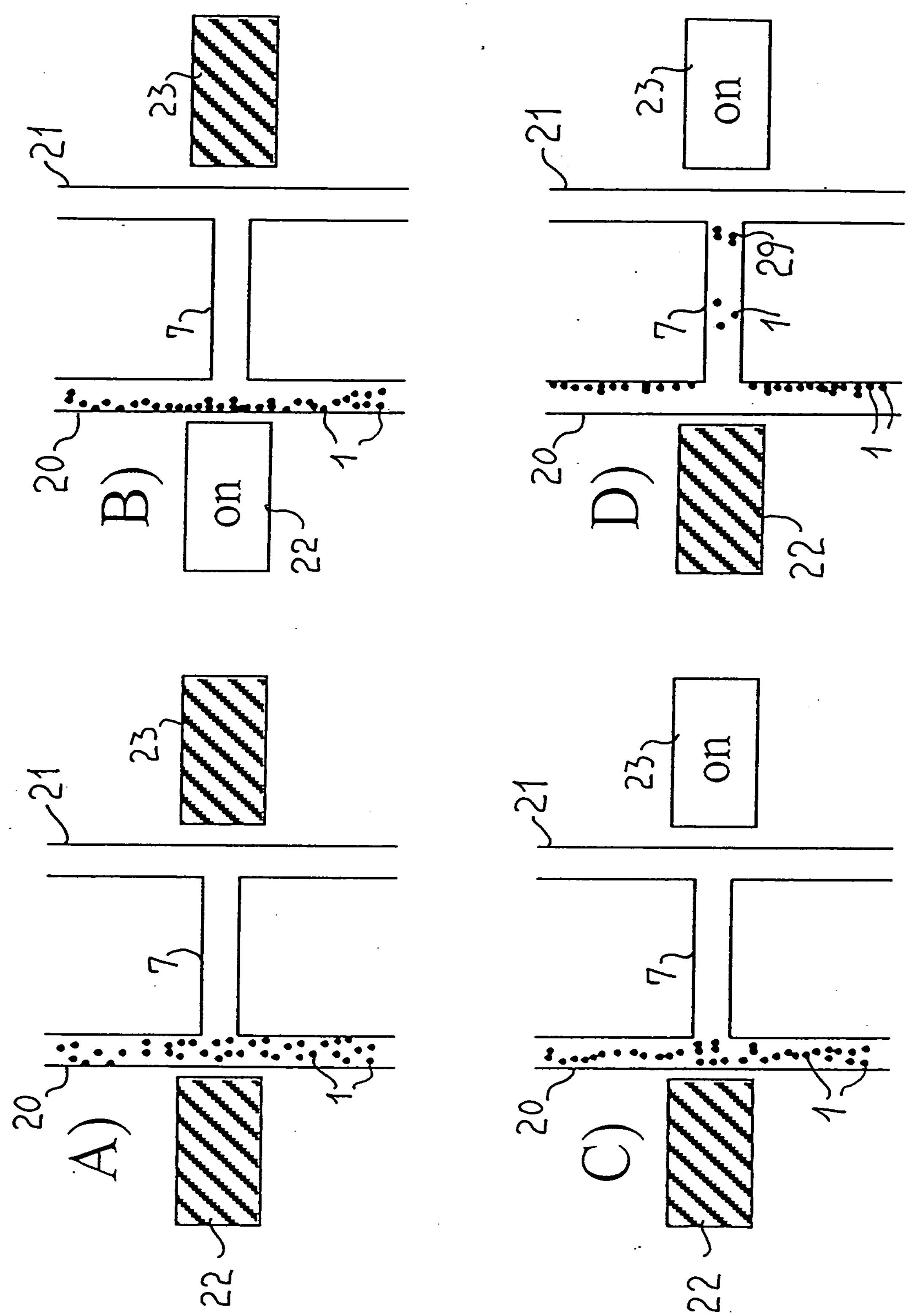


Fig. 6

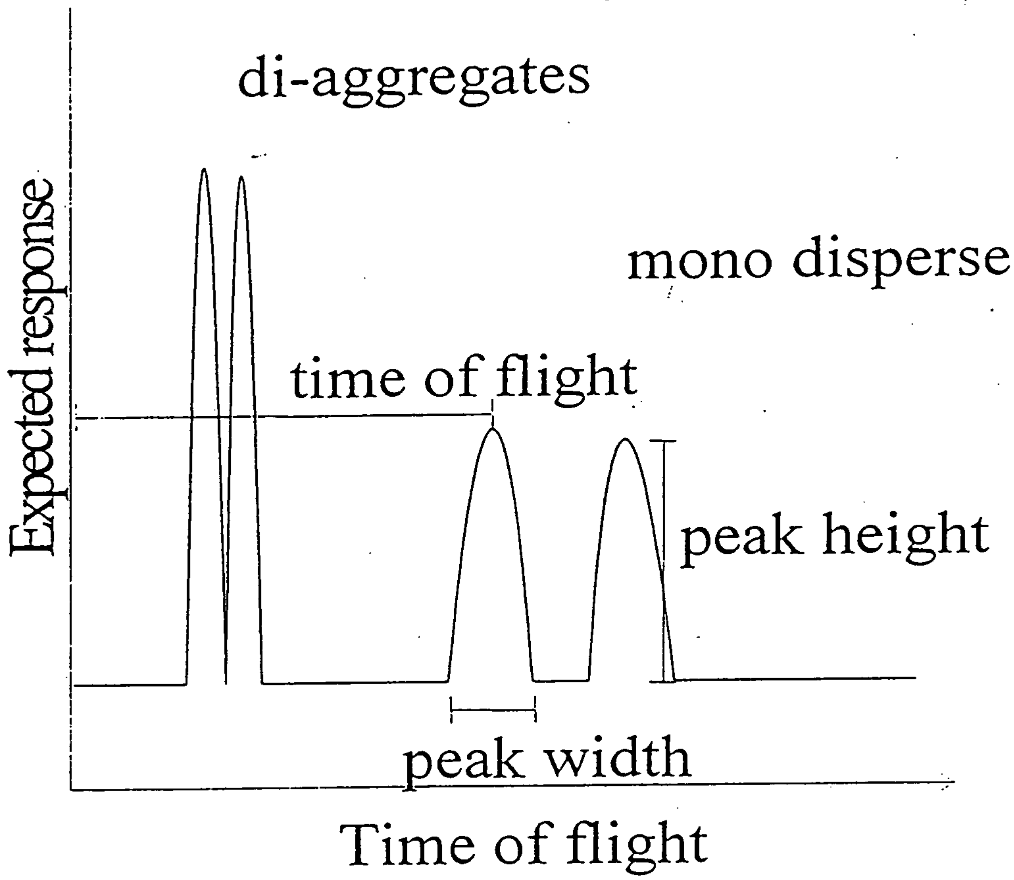

di-aggregates
Expected response
mono disperse
time of flight
peak height
peak width
Time of flight

Fig. 7

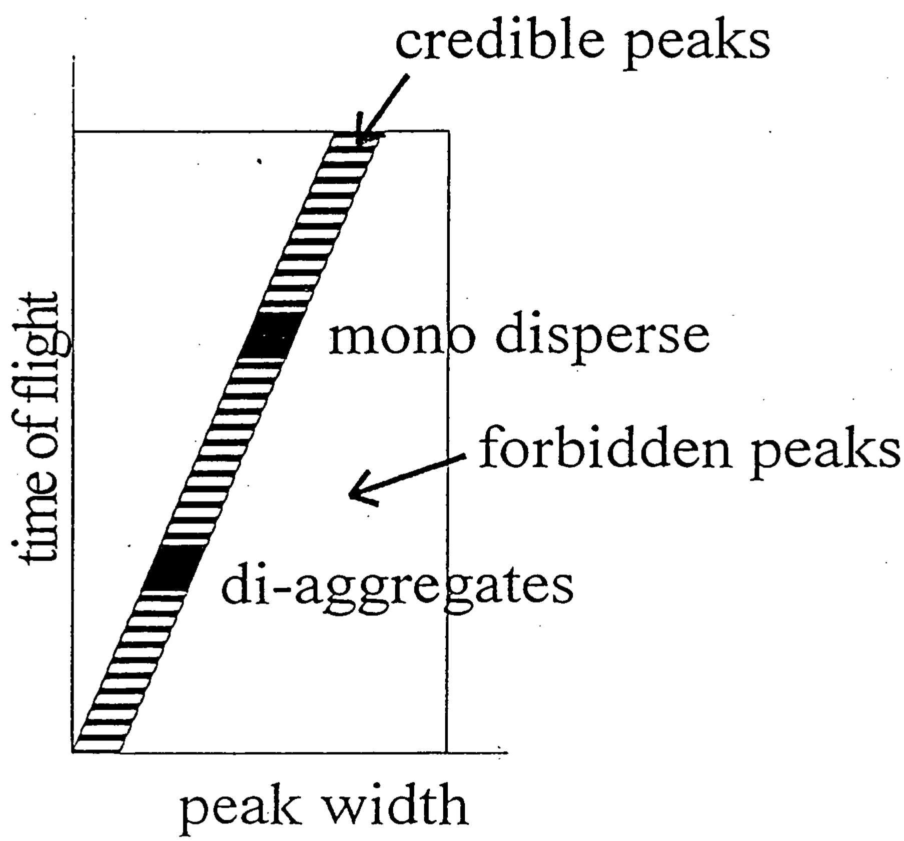

credible peaks
time of flight
mono disperse
forbidden peaks
di-aggregates
peak width

Fig. 8

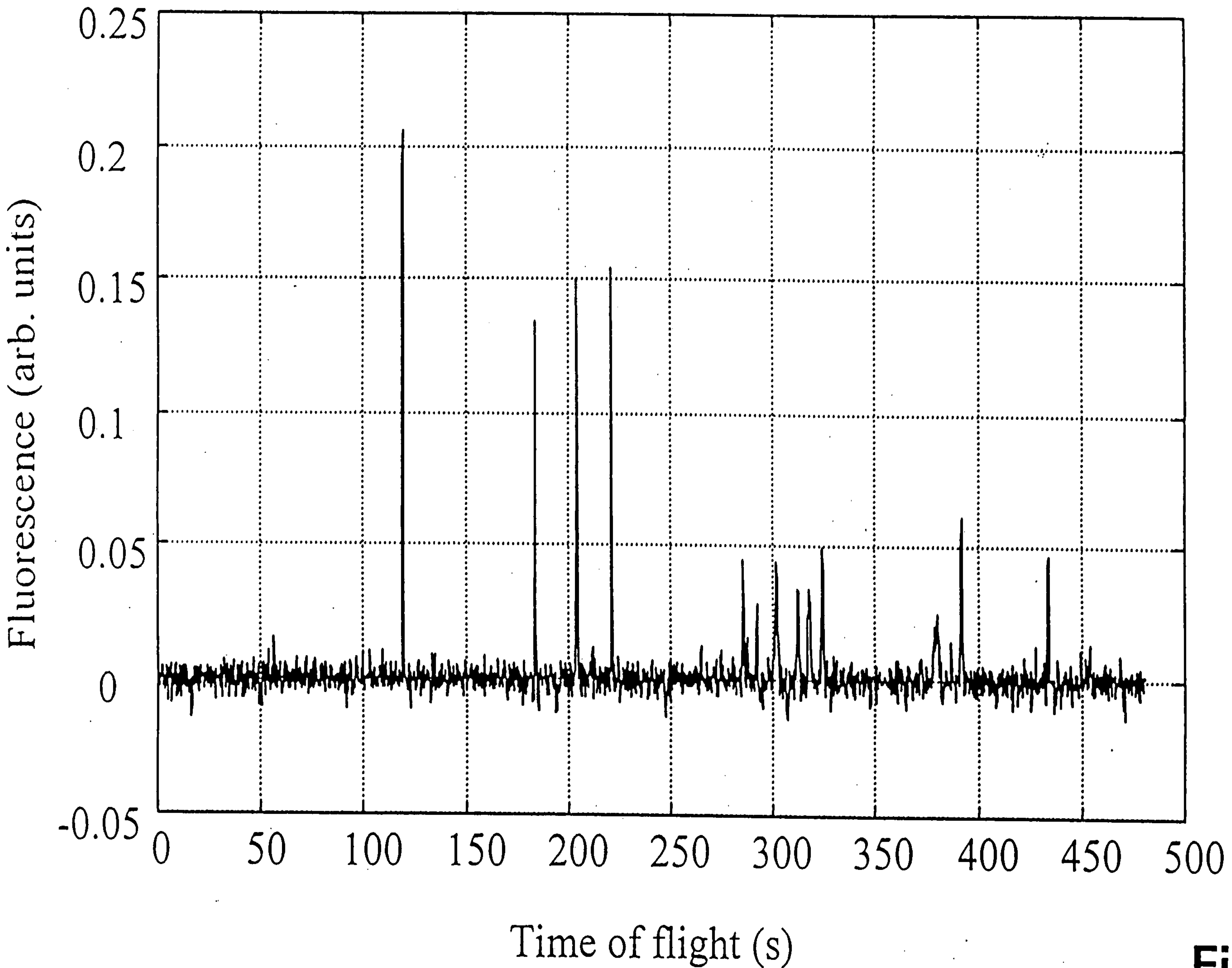

0.25
0.2
0.15
0.1
0.05
0
-0.05
Fluorescence (arb. units)
0
50
100
150
200
250
300
350
400
450
500
Time of flight (s)

Fig. 9

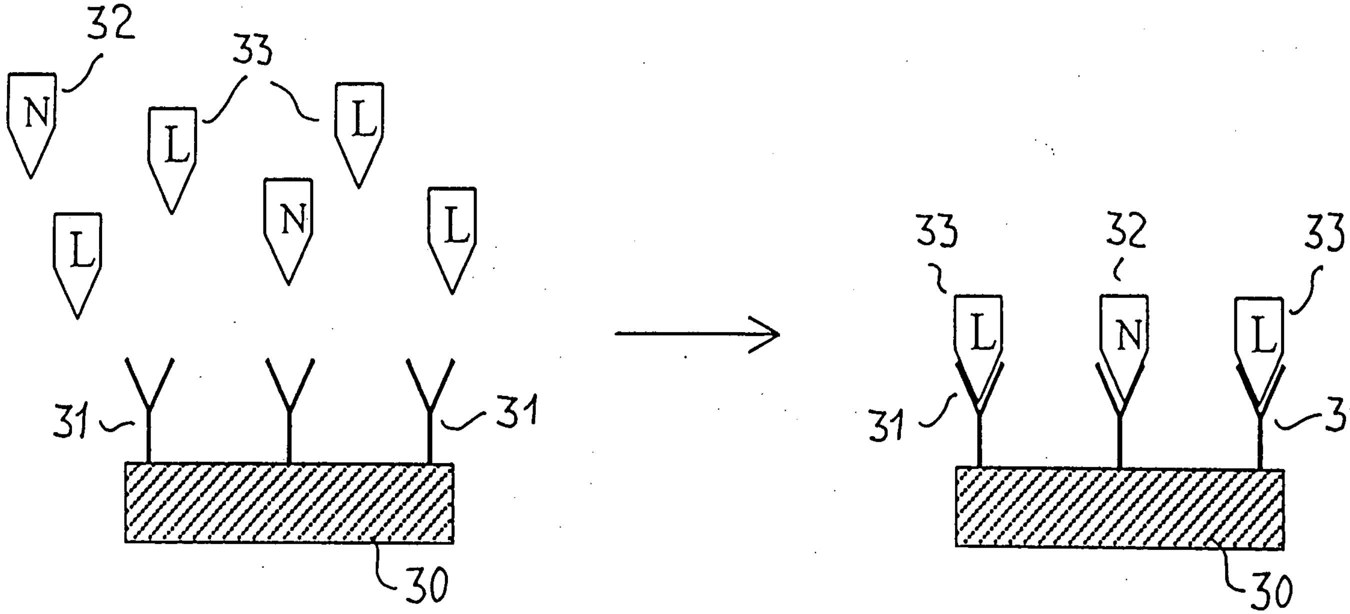


Fig. 10a

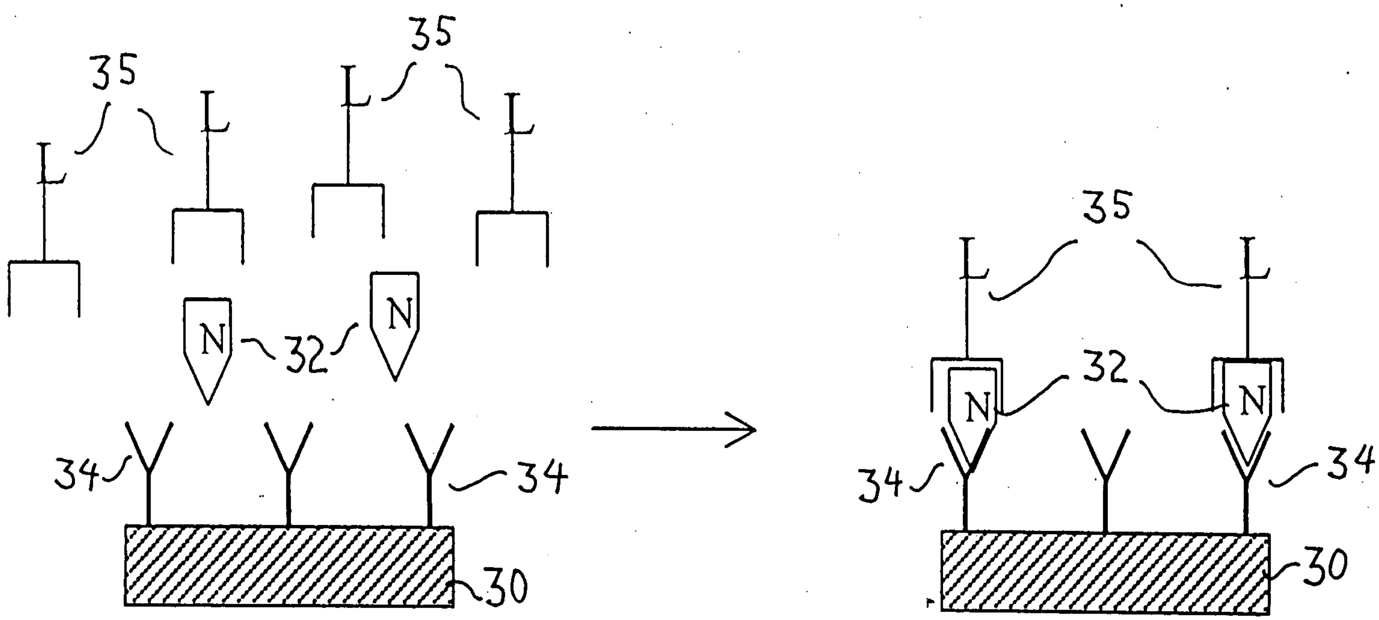


Fig. 10b

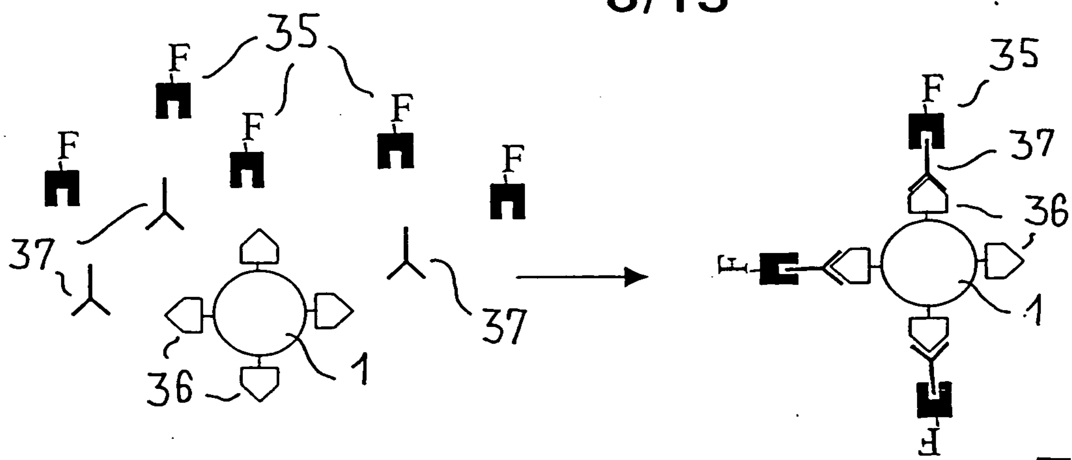


Fig. 11

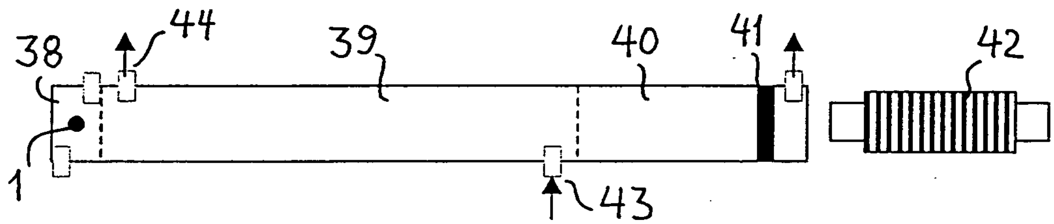


Fig. 12a

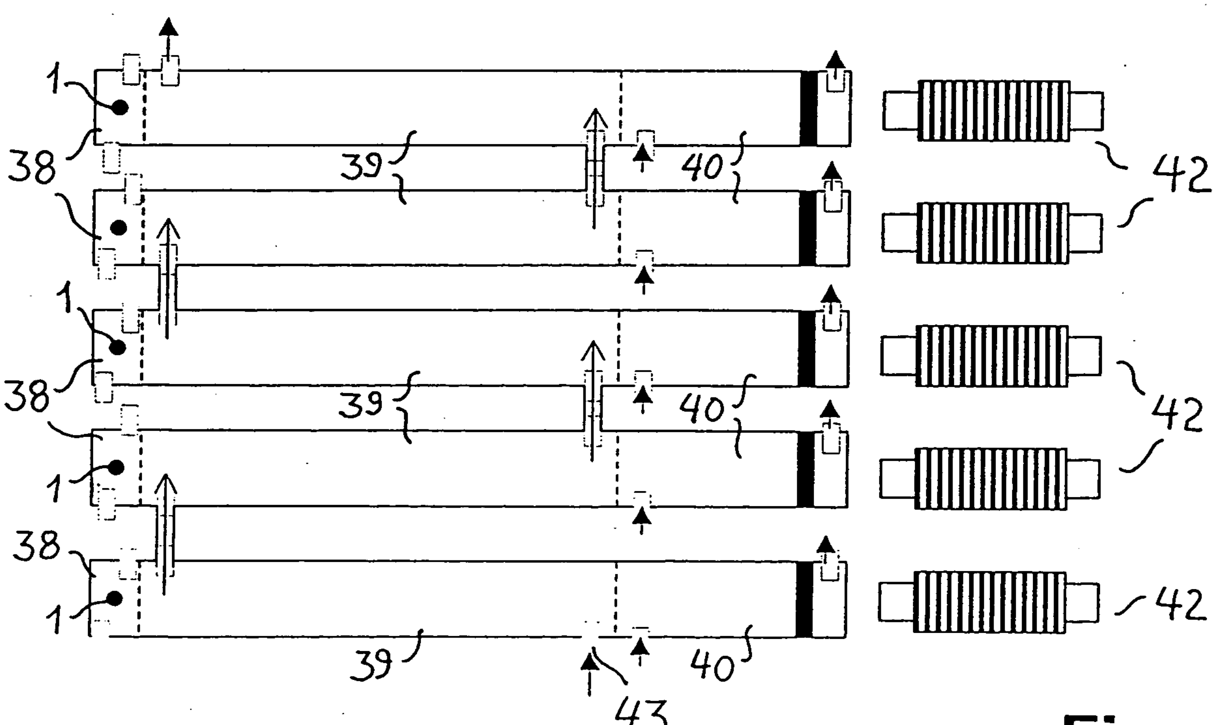


Fig. 12b

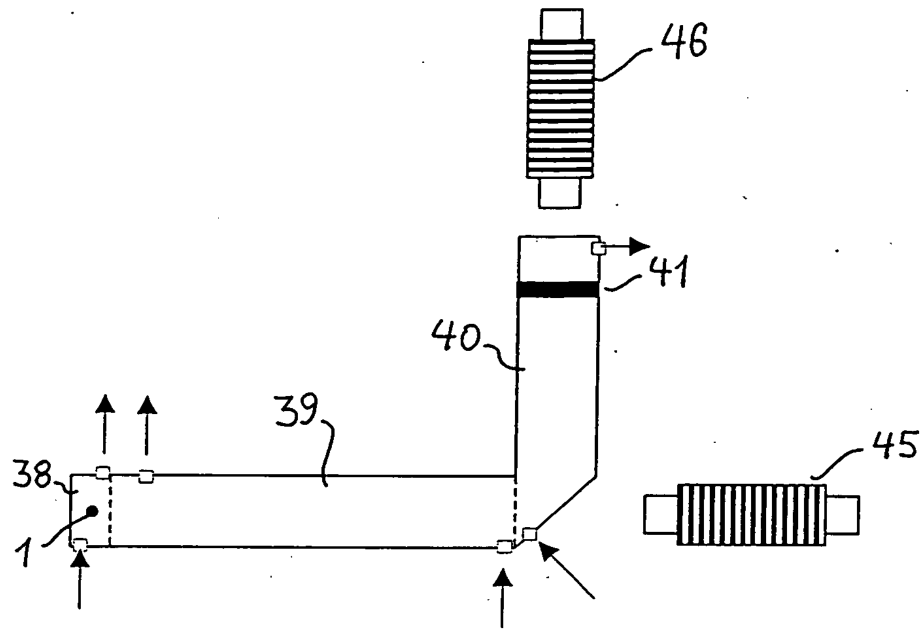


Fig. 13

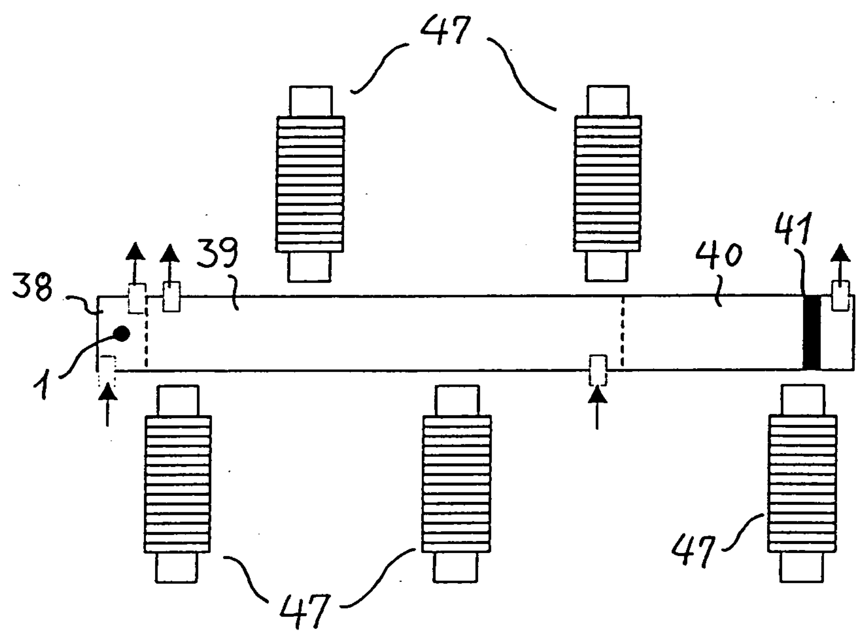


Fig. 14

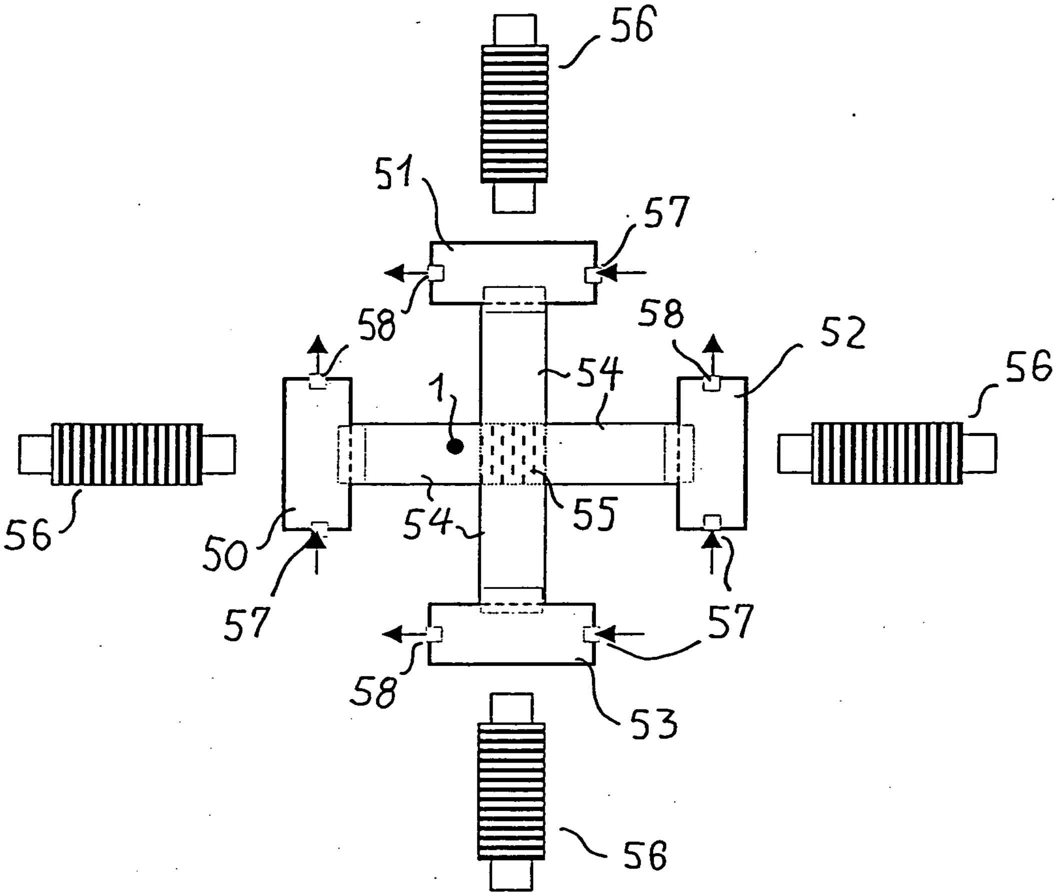
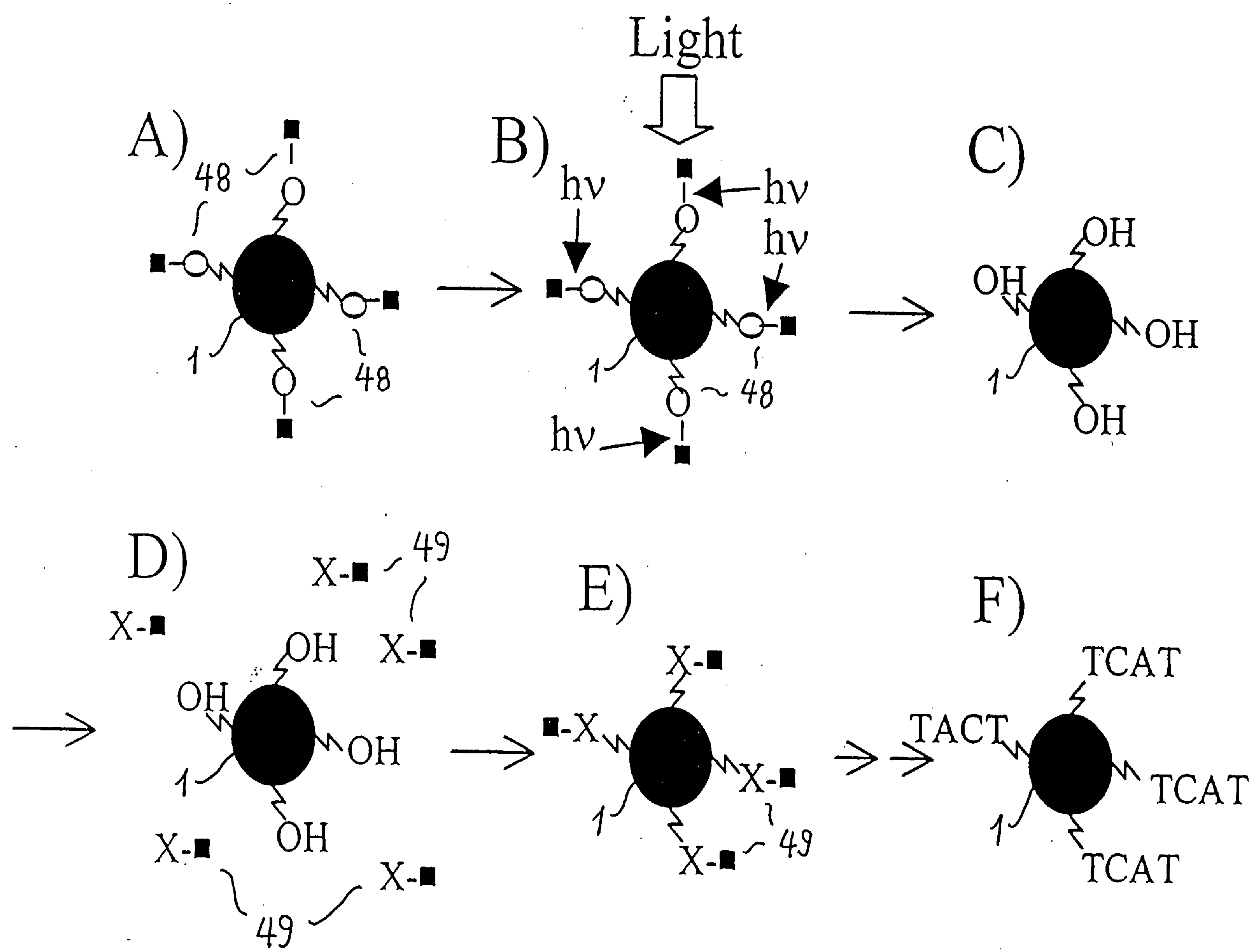


Fig. 15



A)
48
1
48
B)
Light
hν
hν
hν
hν
1
48
C)
OH
OH
OH
OH
1
D)
X-■
X-■
X-■
X-■
X-■
49
49
OH
OH
OH
OH
1
E)
■-X
X-■
X-■
X-■
1
49
F)
TACT
TCAT
TCAT
TCAT
1

Fig. 16

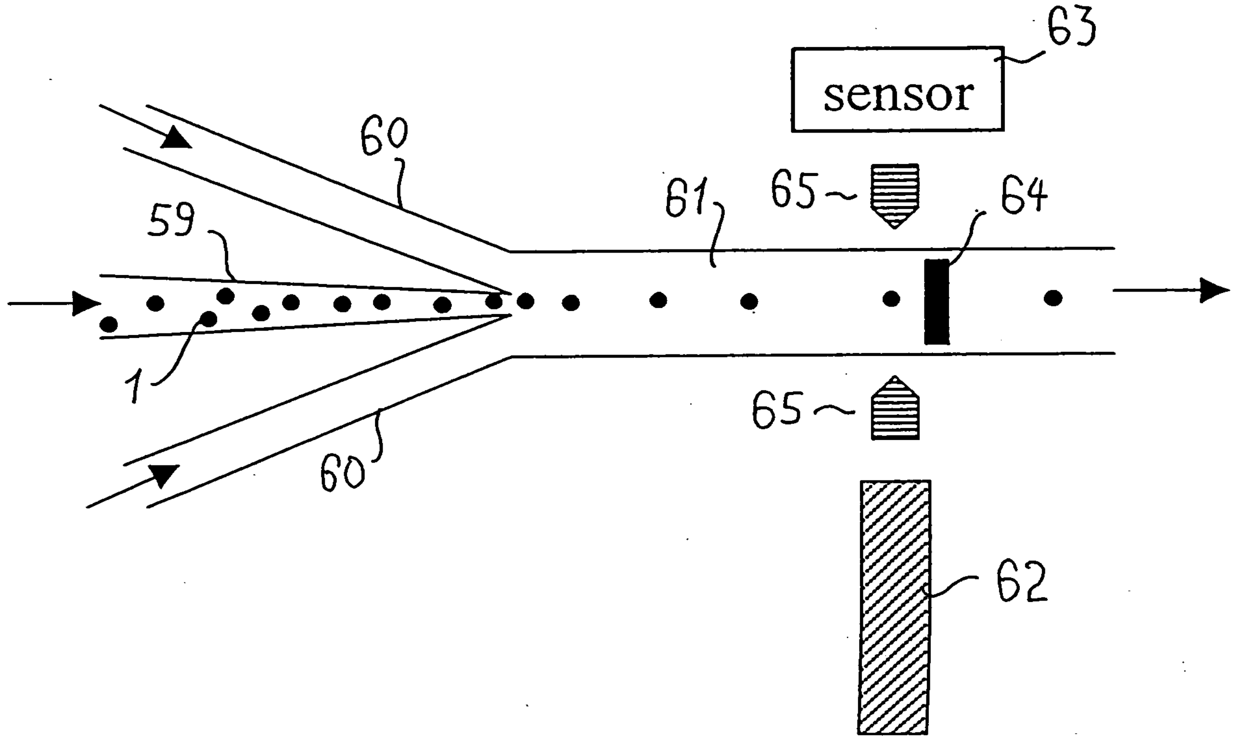


Fig. 17

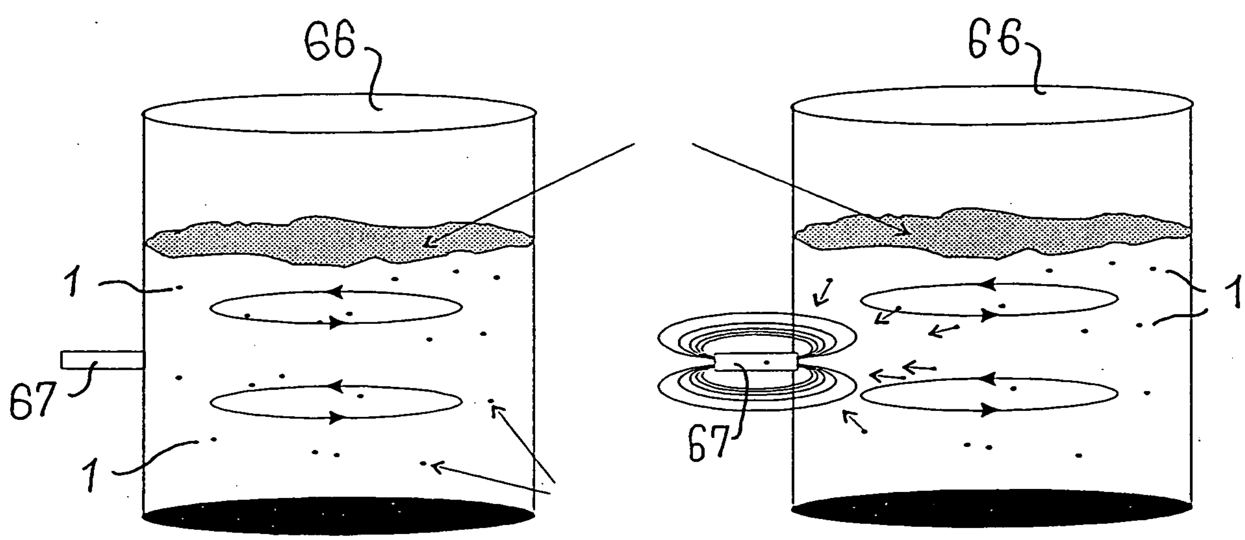


Fig. 18

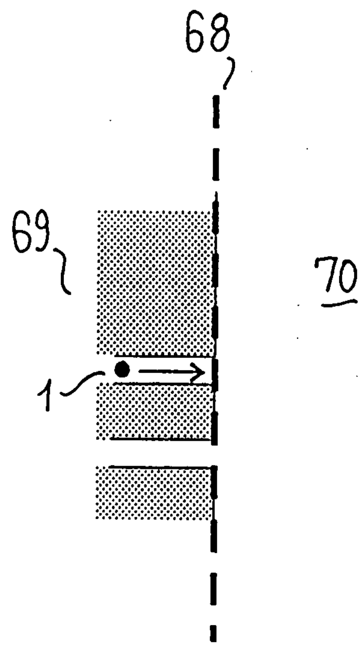


Fig. 19a

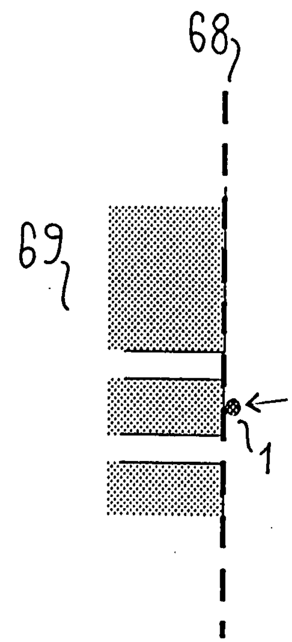


Fig. 19b

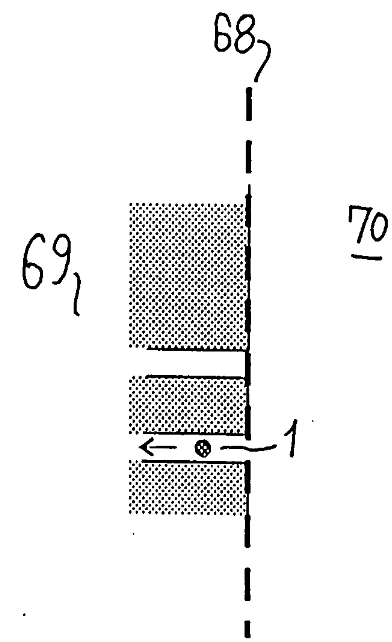


Fig. 19c

THIS PAGE BLANK (USPTO)